Tulathromycin Solution for Parenteral Injection
For Treatment Of Bovine And Swine Respiratory Diseases

Microbiological Effects on Bacteria of Human Health Concern

A Qualitative Risk Estimation

As part of the New Animal Drug evaluation process for antimicrobial agents in livestock, the FDA/CVM may require a review by the Veterinary Medicine Advisory Committee. This document provides a public record of the Risk Estimation conducted by the Sponsor for injectable tulathromycin for therapeutic use in cattle and swine in accordance with FDA/CVM Guidance #152, entitled "Evaluating the Safety of tulathromycin with regard to its Microbiological Effects on Bacteria of Human Health Concern". This document is submitted as a part of the Human Food Safety Assessment. It summarizes proprietary data and information developed and generated by the Sponsor, as well as published data and information relevant to the Risk Estimation for the proposed therapeutic use of injectable tulathromycin in cattle and swine, consistent with Guidance #152.

September 15, 2004

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ABBREVIATIONS

ADME	Absorption, distribution, metabolism, elimination
ATCC	American Type Culture Collection
BRD	Bovine respiratory disease
CDC	United States Centers for Disease Control
CFU	Colony forming units
ERS	United States Department of Agriculture Economic Research Service
FDA/CVM	United States Food & Drug Administration Center for Veterinary Medicine
FSIS	United States Department of Agriculture Food Safety Inspection Service
Guidance #152	United States Food & Drug Administration Center for Veterinary Medicine Guidance #152 entitled, "Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern" [1]
MAC	*Mycobacterium avium* complex
MAI	*Mycobacterium avium-intracellulare*
MPN	Mean probable number
MBC	Minimal bactericidal concentration
MIC	Minimal inhibitory concentration
MLS_B	Macrolide-lincosamide-streptogramin-component B
NAHMS	United States National Animal Health Monitoring System
NARMS	United States National Antimicrobial Resistance Monitoring System
NCCLS	National Committee for Clinical Laboratory Standards
PAE	Post-antibiotic effect
RNA	Ribonucleic acid
SRD	Swine respiratory disease
USDA	United States Department of Agriculture

GLOSSARY OF TERMS

The following terms have been defined by the US Food & Drug Administration Center for Veterinary Medicine (FDA/CVM) in the Guidance for Industry #152 (Guidance #152), entitled, "Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern" [1]. The definitions below are copied verbatim from Guidance #152 and are the definitions used in this document.

Consequence assessment: The consequence assessment describes the relationship between specified exposures to a biological agent (the hazardous agent) and the consequences of those exposures. For the purposes of this risk assessment, FDA has decided that the potential human health consequences of exposure to the defined hazardous agent may be estimated qualitatively by considering the human medical importance of the antimicrobial drug in question.

Exposure assessment: The exposure assessment describes the likelihood of human exposure to the hazardous agent through food-borne exposure pathways. The exposure assessment should estimate qualitatively the probability of this exposure to bacteria of human health concern through food-related pathways.

Hazard: Human illness, caused by an antimicrobial-resistant bacteria, attributable to an animal-derived food commodity, and treated with the human antimicrobial drug of interest.

Hazardous agent: Antimicrobial-resistant food-borne bacteria of human health concern that are in or on a food-producing animal as a consequence of the proposed use of the antimicrobial new animal drug.

Hazard characterization: The process by which one may identify the hazard and the conditions that influence the occurrence of that hazard. This is based upon drug-specific information, bacteria/resistance determinant information, and the methodology for the determination of “resistant” or “susceptible” bacteria.

Release assessment: The release assessment should describe those factors related to the antimicrobial new animal drug and its use in animals that contribute to the emergence of resistant bacteria or resistance determinants (i.e., release of the hazardous agent) in the animal. The release assessment should also estimate qualitatively the probability that release of the hazardous agent would occur. For the purposes of this assessment process, the boundaries of the release assessment span from the point the antimicrobial new animal drug is administered to the food-producing animal, to the point the animal is presented for slaughter or the animal-derived food is collected.

Risk: The probability that human food-borne illness is caused by a specified antimicrobial-resistant bacteria, is attributable to a specified animal-derived food commodity, and is treated with the human antimicrobial drug of interest.

Risk estimation: The overall estimate of the risk associated with the proposed use of the drug in the target food-producing animals following the integration of the release assessment,

exposure assessment and consequence assessment. The risk rankings represent the relative potential for human health to be adversely impacted by the emergence of antimicrobial resistance associated in a food-borne pathogen with the use of the drug in food-producing animals

EXECUTIVE SUMMARY

Tulathromycin is a macrolide antimicrobial agent proposed for therapeutic use in treatment of swine respiratory disease (SRD) and bovine respiratory disease (BRD). It is formulated for parenteral injection as a single dose to provide a full course of therapy, and will be available only by veterinary prescription.

Tulathromycin has a semi-synthetic, 15-membered macrolide ring structure. It is a member of the triamilide subclass of macrolides because it has three polar amine groups, which distinguish its structure from other macrolides, including the azalides and ketolides. Biochemical, microbiological, genetic and molecular studies have been reviewed and accepted by the FDA/CVM that are relevant to understanding: 1) the mechanism of action of tulathromycin, 2) the activity of tulathromycin against macrolide-sensitive and resistant organisms, 3) cross-resistance mechanisms and 4) potential of tulathromycin to select for resistance development in foodborne pathogens of bacteria associated with livestock.

Despite the unique structural features and pharmacokinetic properties of tulathromycin, the bacterial mechanisms of resistance to this molecule are consistent with those applicable to macrolides approved for various uses in livestock for the past 30 years. Furthermore, the FDA/CVM has reviewed and accepted data supporting that tulathromycin has a cross-resistance profile comparable to that observed for the older macrolides used in animal medicine, but *not* like the ketolide subclass of macrolides more recently introduced for use in human medicine. Tulathromycin has minimal inhibitory effect on protein synthesis by erythromycin-resistant ribosomes. Furthermore, tulathromycin is not active against macrolide-resistant bacteria (notably macrolide-resistant *Streptococcus pneumoniae*) that are susceptible to telithromycin, a member of the ketolide subclass of macrolides.

Hazard Analysis Summary

Tulathromycin has some *in vitro* activity against *E. coli*, *Salmonella* and *Enterococcus* under standardized *in vitro* test conditions. However, the activity against these organisms is pH dependent and substantially diminished at the neutral to acidic pH that occurs in the colonic contents and feces of animals. Thus, tulathromycin has low potential to select for resistant organisms or resistance determinants the colonic content and in feces.

Macrolides do not have label indications for treatment of infectious disease due to *E. coli* or *Salmonella* in humans; most macrolides do not have appreciable activity *in vitro* against these organisms; neither quality control performance standards nor clinical susceptibility breakpoints have been generated for macrolides for these organisms; and macrolide resistance is not a component of national surveillance or resistance of clinical isolates from humans. Furthermore, macrolides are not a drug of choice for treatment of enterococcal infections, due in part to the high macrolide resistance rates in *Enterococcus* species documented since the 1970's [2]. For these reasons, macrolide resistance in *E. coli, Salmonella,* and *Enterococcus* is not addressed in this risk estimation.

Macrolides can be used for treatment of campylobacteriosis in patients requiring antimicrobial therapy. Therefore the risk of campylobacteriosis requiring macrolide therapy is considered in accordance with Guidance #152 [1] as follows:

THE HAZARD **(defined by Guidance #152 as "Human illness, caused by an antimicrobial-resistant bacteria, attributable to an animal-derived food commodity, and treated with the human antimicrobial drug of interest")** being considered is campylobacteriosis, caused by a macrolide-resistant *Campylobacter*, attributable to a food commodity derived from swine or cattle, and treated with a macrolide.

THE HAZARDOUS AGENT (**defined by Guidance #152 as "Antimicrobial-resistant food-borne bacteria of human health concern that are in or on a food-producing animal as a consequence of the proposed use of the antimicrobial new animal drug."**) being considered in this document is: macrolide-resistant food-borne *Campylobacter* that are in or on cattle or swine as a consequence of the proposed use of tulathromycin.

THE SPECIFIC RISK **(defined by Guidance #152 as " The probability that human food-borne illness is caused by a specified antimicrobial resistant bacteria, is attributable to a specified animal-derived food commodity, and is treated with the human antimicrobial drug of interest")** being considered is the probability that campylobacteriosis is caused by a macrolide- resistant *Campylobacter*, is attributable to a bovine- or swine-derived food commodity, and is treated with a macrolide.

Release Assessment Summary

Taking into account the factors outlined in Guidance #152 for the release assessment, there is a "Low" probability that release of macrolide-resistant *Campylobacter* will occur, for several reasons. First, the microbiological activity of tulathromycin is substantially diminished due to the neutral to acidic pH in colonic contents and in feces and binding to fecal substrates [3]. Second, macrolide resistance in *Campylobacter* occurs by a mutational event in *Campylobacter*, not by acquisition of a macrolide resistance gene(s) and thus resistance transfer is not evident in this organism [Section 1.7]. Third, the observed frequency [3] of mutation *in vitro* to tulathromycin- or other macrolide-resistance for *Campylobacter* and other organisms tested (*E. coli*, *Enterococcus* and *Salmonella*) was below the detection limits expected for spontaneous mutation [4,5]. Fourth, no unique mechanisms of macrolide resistance have been detected, selected or induced in the presence of tulathromycin in studies reviewed and accepted by the FDA/CVM [3]. Fifth, the proposed use of tulathromycin is consistent with appropriate judicious use principles of veterinary medicine. Tulathromycin will be administered by parenteral injection, under veterinary prescription only, to individual animals requiring treatment due to bacterial respiratory disease (cattle and swine) or at known high risk for bacterial respiratory disease (cattle). It is not intended for whole herd medication. The proposed single dose regimen provides a full course of therapy, which diminishes the likelihood of recrudescence occurring due to non-compliance by the user. A single dose regimen also reduces overall stress to the production animal associated with repeated drug administration. The product is intended for use consistent with judicious use principles for a therapeutic antibacterial in cattle and swine. Finally, bacterial respiratory

disease is most frequently encountered in animals, well before the animal is to be shipped from the production site for slaughter, packaging, and entry into the retail food chain.

Three macrolides (erythromycin, tylosin, and tilmicosin) are currently approved for use in cattle and swine. Erythromycin is approved for use in cattle and swine as an injectable formulation. Tylosin is approved for use in swine and cattle as both an injectable formulation and as a feed premix. Tilmicosin is approved for use in cattle as an injectable formulation and as a feed premix in swine. Tulathromycin will be available only as an injectable formulation. The selection pressure exerted by tulathromycin is not expected to contribute substantially to that of macrolide products currently available for use in livestock.

To date, macrolide resistance in *Campylobacter* appears to occur as a result of a mutational event, and not from a gene transfer event (Sections 1.7.1.1,1.7.2.3). The 1997-2001 data generated from the US Centers for Disease Control (CDC) National Antimicrobial Resistance Monitoring System (NARMS) program for enteric bacteria [6] show that the prevalence of macrolide resistance is only between 1-3% among *Campylobacter* isolates from humans, with no apparent trends over time and despite macrolide use in swine, cattle and poultry for over 30 years.

Therefore, the release assessment is qualitative ranked as a "Low" probability that release of a macrolide-resistant *Campylobacter* would occur.

Exposure Assessment Summary

Guidance #152 states, "The exposure assessment describes the likelihood of human exposure to the hazardous agent through food-borne exposure pathways. The exposure assessment should estimate qualitatively the probability of this exposure to bacteria of human health concern through food-related pathways."

In this exposure assessment, the likelihood of human exposure to macrolide-resistant *Campylobacter* through the food-borne route uses the algorithm outlined in Table 5 of Guidance #152, copied in Figure 1 to integrate the ranking of food consumption and *Campylobacter* contamination.

Figure 1 CVM Guidance #152 Table 5 [1]: Possible process for ranking qualitatively the probability of human exposure to *Campylobacter* in a given food commodity:

	Probability of human exposure to a given bacteria		
	Amount of food commodity (beef or swine) being consumed		
Amount of food commodity contamination	**High**	**Medium**	**Low**
High	**H**	**H**	**M**
Medium	**H**	**M**	**L**
Low	**M**	**L**	**L**

Cattle. According to Guidance #152 [1], the Exposure Assessment describing the likelihood of human exposure to macrolide-resistant *Campylobacter* through consumption of beef can be assigned by integrating the US national beef consumption data (62.9 pounds/capita/year) listed in Table 2 of Guidance #152 and ranked as "High", with *Campylobacter* contamination rate data for cattle carcass and ground meat (0-4%) listed in Guidance #152 Table 4 and ranked as "Low".

Applying the "High" beef consumption ranking and the "Low" *Campylobacter* contamination ranking to the algorithmic process described in Table 5 in Guidance #152 ([1]; copied in Figure 1 of this document), the Exposure Assessment for human exposure to *Campylobacter* through beef consumption is qualitatively ranked as "Medium".

Swine. According to Guidance #152, the exposure assessment describing the likelihood of human exposure to macrolide-resistant *Campylobacter* through consumption of pork meat can be assigned by integrating US national pork meat consumption data (46.7 pounds/capita/year) listed in Table 2 of Guidance #152 and ranked as "High" [1]) and using the *Campylobacter* contamination rate data for pork carcasses (32%) listed in Table 5 of Guidance #152 [1] and ranked as "High". However, the Sponsor proposes that the contamination rate of pork carcasses is *not* representative of *Campylobacter* contamination rates of pork meat. Contamination rates of pork meat are consistently much lower $\leq$ 5% than that of pork carcasses in the literature (see Section 2.4.3.5). Moreover, contamination rates were 1% for pork chops in the 2002 and 2003 NARMS Retail Meat Surveillance Program [7]. Thus, the contamination rate for pork meat should be qualitatively ranked as "Low".

Applying the "High" pork consumption ranking and the "Low" *Campylobacter* contamination ranking of pork meat ($\leq$5%), to the algorithmic process described in Table 5 in Guidance #152 ([1]; copied in Figure 1 of this document), the Exposure Assessment for human exposure to *Campylobacter* through pork consumption is qualitatively ranked as "Medium".

Consequence Assessment Summary

The Consequence Assessment for macrolide use in human medicine is "Critically Important " because macrolides are used for treatment of the foodborne pathogen, *Campylobacter,* associated with food-producing animals and because they are important for use in treatment of Legionnaire's disease, and prophylaxis and therapy for serious disease due to *Mycobacterium avium* Complex (MAC)/*Mycobacterium avium-intracellulare* (MAI), as presented in Appendix A of Guidance #152 [1].

Overall Qualitative Risk Estimation is "High" for all "Critically Important" Drugs

Table 8 of Guidance #152 shows that all drugs ranked as "Critically Important" also have an overall ”High" Risk Estimation, regardless of exposure or release assessment.

Approval and Management Considerations

There are inherent characteristics of tulathromycin and its proposed use that lower the concern for selection of macrolide-resistant *Campylobacter*. First, tulathromycin activity is attenuated by the pH and binding in the colonic contents and feces. Second, macrolide resistance occurs in *Campylobacter* by chromosomal mutation and not by gene acquisition, the latter of which is of greater concern for gut flora. Third, macrolide resistance in *Campylobacter* isolates from humans has remained $\leq$ 3% with no obvious trends over time in the NARMS surveys [6], after more than thirty years of parenteral, oral, and topical use of macrolides use in humans, companion animals and food animals. Fourth, the prevalence of *Campylobacter* in pork and beef at retail is low (0-5%, Section 2.4). Fifth, the microbiological, molecular, epidemiological, and historical database strongly supports that pork and beef are not significant risk factors for *Campylobacter* causing disease in humans (Section 2.2).

The extent of use limitations listed in Table 7 of Guidance #152 (reproduced in Figure 2) suggest that the proposed extent of use is considered "Low", if the drug is intended for administration to individual animals, *and* the duration of effective dose is $\leq$21 days. This is the case of tulathromycin. Tulathromycin is formulated only for parenteral injection and therefore must be administered to individual animals.

Figure 2. Guidance #152 Table 7 [1]: Possible process for ranking (High, Medium, Low) of extent of antimicrobial drug use in animals based on duration and method of administration.

Duration of use	Intended administration to:		
	Individual animals	**Select groups or pens of animals**	**Flocks or herds of animals**
Short (<6 days)	L[1]	M[2]	H[3]
Medium (6-21 days)	L	M	H
Long (>21 days)	M	H	H

[1]Low, [2]Medium, and [3]High extent of use

Table 8 of Guidance #152 [1] lists examples of potential risk management steps for "Category I" Drugs having an overall "High" Risk Estimation. These steps may include any one or a combination of the following considerations: 1) prescription status, 2) low extent of use, 3) post approval monitoring (e.g., through NARMS), 4) advisory committee review considerations and(or) 5) extra-label use restrictions. The Sponsor proposes that: 1) tulathromycin be approved under veterinary prescription; 2) the extent of use is inherently low based on the proposed use and parenteral single dosage; 3) this document is submitted as a component of the Veterinary Medicine Advisory Committee review; 4) the existing NARMS program provides monitoring for macrolide resistance in *Campylobacter* and 5) no extra-label use restrictions should apply. The Sponsor proposes that extra-label use restrictions are not required for approval, because macrolides have been and are approved for

therapeutic, safe use for a variety of indications in poultry, swine, cattle and other animal species.

CONCLUSION

With respect to microbial safety considerations, the proposed label uses of tulathromycin include management considerations of prescription status, inherent low extent of use due to parenteral single dose administration, and Advisory Committee review. Macrolide resistance in *Campylobacter* is currently monitored in the NARMS program. Therefore, with these management considerations, approval of the proposed indications for injectable tulathromycin in cattle and swine poses no appreciable risk to public health with respect to microbial food safety.

HAZARD CHARACTERIZATION

Tulathromycin is a semi-synthetic, 15-membered ring macrolide containing three polar amine groups. It is a member of the triamilide subclass of macrolides, distinguishing it from other macrolides, including azalides and ketolides.

The FDA/CVM has reviewed and accepted studies documenting that tulathromycin has a mechanism of action and cross-resistance profile that closely match those of erythromycin and tilmicosin as described in Sections 1.4 and 1.6. Tulathromycin is a protein synthesis inhibitor like other macrolides [3; Section 1.3.1]. It competes with erythromycin for binding sites on macrolide-sensitive ribosomes and inhibits protein synthesis in a transcription/translation assay of 30S ribosomal subunit preparations [3]. Strains resistant to erythromycin are also resistant to tulathromycin, with the following notable exception. Unlike erythromycin, tulathromycin (like tilmicosin) is a comparatively weak inducer of macrolide resistance due to *erm*B [3].

There is extensive cross-resistance among tulathromycin, tilmicosin and erythromycin as demonstrated by the minimal inhibitory concentrations (MIC) of these drugs tested against gram-positive human pathogens with known macrolide-resistance determinants [3]. However, tulathromycin is not active against macrolide-resistant *S. pneumoniae* that are susceptible to the ketolide, telithromycin, used in human medicine. Therefore, the activity of ketolides and their use in man should not be compromised by tulathromycin use in animals.

In human medicine in the United States, macrolides are not used for treatment of infections due to *Salmonella* or *E. coli*. NCCLS does not list quality control performance standards or susceptibility breakpoint criteria for erythromycin, azithromycin or clarithromycin for these gram-negative enteric organisms [8]. The NARMS program does not monitor MICs or susceptibility of *Salmonella* isolates from humans [6].

In humans, macrolides are used primarily for treatment of respiratory tract infections (bronchitis, pneumonia, pharyngitis, sinusitis, ear infections), and to a lesser extent for skin and urinary tract infections caused by *Staphylococcus* and *Streptococcus* species. They are also used to treat infections due to atypical organisms such as *Legionella pneumophilia*, *Mycoplasma pneumoniae, Chlamydia pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis, Mycobacterium avium* complex and *Mycobacterium avium-intracellulare.* These organisms are spread among humans via respiratory droplets and other secretions, and acquisition of these disease-producing organisms by foodborne routes has not been noted in the literature [9,10,11,12,13,14]. Guidance #152 states that the risk estimation represents the relative potential for human health to be adversely impacted by the emergence of antimicrobial resistance associated in a food-borne pathogen with use of a drug in food-producing animals. Thus these organisms are not the subject of this document.

Macrolides are not generally used as therapeutic agents for treatment of nosocomial *Enterococcus* infections, due to their limited activity against these organisms. The NCCLS does not recommend routine (primary) testing of macrolides against Group A, Group B and

Group U enterococci, and suggest selective, supplemental reporting of Group C enterococci from humans [8].

There are alternatives to conventional macrolides for treatment of infections caused by respiratory pathogens and enterococci. Ketolides retain activity against many types of erythromycin-resistant organisms [15,1617,18]. Other drug classes include streptogramins (e.g., quinupristin/dalfopristin) and oxazolidones (e.g., linezolid) both of which act by inhibiting protein synthesis. The latter drug classes do not produce cross-resistance to conventional macrolides such as erythromycin and azithromycin [19,20] and bind to macrolide-resistant ribosomes. Since the tulathromycin mechanism of action and resistance are characteristic of the conventional macrolides, bacterial cross-resistance to the latter drug classes is not expected or likely.

There are concerns that use of macrolides in livestock can increase the pool of transferable macrolide resistance determinants in *Enterococcus* which may ultimately affect human therapeutic needs for treatment of vancomycin-resistant and streptogramin-resistant *E. faecium* [21,22]. While tulathromycin is active against *Enterococcus* species *in vitro*, its activity is highly attenuated at the neutral to acidic pH normally encountered in colonic contents and in feces (See Section 1.4.2). At these pH values, the activity of tulathromycin toward these organisms is substantially attenuated, limiting the potential for tulathromycin to exert selective pressure for resistance development or transfer among these organisms. Furthermore, studies show that tulathromycin binding occurs when tulathromycin is added to fecal slurries (Section 1.5.1.3).

Considering this information, this risk estimation focuses on campylobacteriosis caused by macrolide-resistant *Campylobacter*. Tulathromycin is active *in vitro* against *Campylobacter*. Macrolides and fluoroquinolones are the drugs typically used to treat campylobacteriosis in patients in need of antimicrobial therapy. Currently, macrolide resistance in *Campylobacter* isolated from humans is low. In the NARMS reports [6], the prevalence of macrolide resistance among *Campylobacter* isolates from humans was between 1 and 3% from 1997-2001 [6], with no discernable trends over time.

From the available information, and in context of Guidance #152 [1], the qualitative risk assessment for tulathromycin addresses the following:

THE HAZARD **(defined by Guidance #152 as "Human illness, caused by an antimicrobial-resistant bacteria, attributable to an animal-derived food commodity, and treated with the human antimicrobial drug of interest")** being considered is campylobacteriosis, caused by a macrolide-resistant *Campylobacter*, attributable to a food commodity derived from swine or cattle, and (the patient is) treated with a macrolide.

THE HAZARDOUS AGENT (**defined by Guidance #152 as "Antimicrobial-resistant food-borne bacteria of human health concern that are in or on a food-producing animal as a consequence of the proposed use of the antimicrobial new animal drug."**) being

considered in this document is: macrolide-resistant food-borne *Campylobacter* that are in or on cattle or swine as a consequence of the proposed use of tulathromycin.

THE SPECIFIC RISK **(defined by Guidance #152 as " The probability that human food-borne illness is caused by a specified antimicrobial resistant bacteria, is attributable to a specified animal-derived food commodity, and is treated with the human antimicrobial drug of interest")** being considered is the probability that campylobacteriosis is caused by a macrolide-resistant *Campylobacter*, is attributable to bovine- or swine-derived food commodity, and is treated with a macrolide.

QUALITATIVE RISK ESTIMATION

1. RELEASE ASSESSMENT

1.1. Overview

Guidance #152 states [1]: "The release assessment should describe those factors related to the antimicrobial new animal drug and its use in animals that contribute to the emergence of resistant bacteria or resistance determinants (i.e., release of the hazardous agent) in the animal. The release assessment should also estimate qualitatively the probability that release of the hazardous agent would occur. For the purposes of this assessment process, the boundaries of the release assessment span from the point the antimicrobial new animal drug is administered to the food-producing animal, to the point the animal is presented for slaughter or the animal-derived food is collected."

Taking into account the factors outlined in Guidance #152 for the release assessment, there is a "Low" probability that release of macrolide-resistant *Campylobacter* will occur, for several reasons. First, the microbiological activity of tulathromycin is substantially diminished due to the neutral to acidic pH in colonic contents and in feces and binding to fecal substrates [3]. Second, macrolide resistance in *Campylobacter* occurs by a mutational event in *Campylobacter*, not by acquisition of a macrolide resistance gene(s) and thus resistance transfer is not evident in this organism [Section 1.7]. Third, the observed frequency [3] of mutation *in vitro* to tulathromycin- or other macrolide-resistance for *Campylobacter* and other organisms tested (*E. coli*, *Enterococcus* and *Salmonella*) was below the detection limits expected for spontaneous mutation [4,5]. Fourth, no unique mechanisms of macrolide resistance have been detected, selected or induced in the presence of tulathromycin in studies reviewed and accepted by the FDA/CVM [3]. Fifth, the proposed use of tulathromycin is consistent with appropriate judicious use principles of veterinary medicine. Tulathromycin will be administered by parenteral injection, under veterinary prescription only, to individual animals requiring treatment due to bacterial respiratory disease (cattle and swine) or at known high risk for bacterial respiratory disease (cattle). It is not intended for whole herd medication. The proposed single dose regimen provides a full course of therapy, which diminishes the likelihood of recrudescence occurring due to non-compliance by the user. A single dose regimen also reduces overall stress to the production animal associated with repeated drug administration. The product is intended for use consistent with judicious use principles for a therapeutic antibacterial in cattle and swine. Finally, bacterial respiratory disease is most frequently encountered in animals, well before the animal is to be shipped from the production site for slaughter, packaging, and entry into the retail food chain.

Three macrolides (erythromycin, tylosin, and tilmicosin) are currently approved for use in cattle and swine. Erythromycin is approved for use in cattle and swine as an injectable formulation. Tylosin is approved for use in swine and cattle as both an injectable formulation and as a feed premix. Tilmicosin is approved for use in cattle as an injectable formulation and as a feed premix in swine. Tulathromycin will be approved only as an injectable formulation. The selection pressure exerted by tulathromycin is not expected to contribute substantially to that of macrolide products currently available for use in livestock.

To date, macrolide resistance in *Campylobacter* appears to occur as a result of a mutational event, and not from a gene transfer event (Section 1.7.1.1,1.7.2.3). The 1997-2001 data generated from the US Centers for Disease Control (CDC) National Antimicrobial Resistance Monitoring System (NARMS) program for enteric bacteria [6] show that the prevalence of macrolide resistance is only between 1-3% among *Campylobacter* isolates from humans, with no apparent trends over time and despite macrolide use in swine, cattle and poultry for over 30 years.

Therefore, the release assessment is qualitative ranked as a "Low" probability that release of a macrolide-resistant *Campylobacter* would occur.

1.2. Product description

1.2.1. Product formulation

Active ingredients: Tulathromycin in solution for parenteral injection

1.2.2. Proposed conditions of use

Route of administration: Parenteral injection in cattle and swine

Dosage regimen: Administered as a single injection at the proposed dose that will provide a full course therapy (cattle or swine)

Proposed Indication: Treatment of bacterial infections due to proposed label pathogens causing bovine respiratory disease (BRD) and control of respiratory disease in cattle at high risk of developing BRD, associated with target pathogens

Treatment of bacterial infections due to proposed label pathogens causing swine respiratory disease (SRD)

Target Animal Species: Swine and beef cattle (not for use in lactating dairy cattle or in pre-ruminant calves)

Proposed withdrawal time: A pre-slaughter withdrawal time will be assigned by FDA/CVM.

1.2.3. Drug substance description

Drug Class: Macrolide

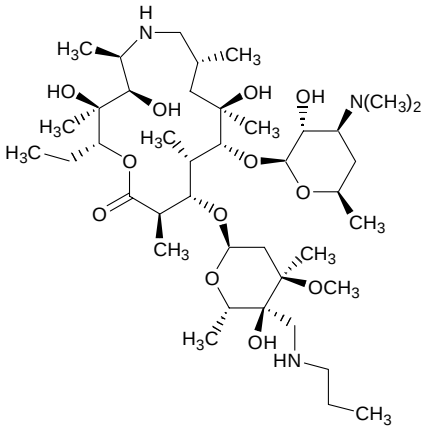
Macrolide subclass Tulathromycin differs from other macrolides since it has three amino groups. Thus it has a chemical subclass designation of triamilide distinguishing its structure from azalides, ketolides, and other macrolides

[23,24]. Tulathromycin is used exclusively for veterinary medicine.

Molecular formula: $C_{41}H_{79}N_3O_{12}$

Molecular weight 806.23

Chemical tructure of tulathromycin



1.3. Mechanism and type of action

1.3.1. Mechanism of action

Macrolides inhibit protein synthesis by binding to bacterial ribosomes, stimulating the dissociation of peptidyl-tRNA from the ribosome during the translocation process [4,25,28]. Translation inhibition studies and ribosomal binding studies of tulathromycin, reviewed and accepted by the FDA/CVM, show that the mechanism of action of the triamilide to involve ribosomal binding, comparable to that observed for other macrolides.

Tulathromycin was tested in transcription-translation assays for protein synthesis by 30S ribosomal subunit preparations from macrolide-susceptible and resistant *E. coli* (Table 1). The inhibitory activity of tulathromycin was comparable to that determined for erythromycin, tilmicosin and clarithromycin. None of these drugs inhibited protein synthesis of 30S preparations from macrolide-resistant ribosomes of *E. coli.*

Table 1. Inhibitory activity of macrolides in a transcription-translation assay using ribosomes isolated from *E. coli

Compound	Mean IC_{50} (μM)† for macrolide-sensitive ribosome (std deviation)	Mean IC_{50} (μM)† for macrolide-resistant ribosome
Tulathromycin	0.44 (0.1)	>150
Erythromycin	0.57 (0.17)	>150
Tilmicosin	0.39 (.04)	>150
Clarithromycin	0.64 (0.14)	>150
Quinupristin/dalfopristin	0.99 (0.05)	0.53

*Determinations with erythromycin-sensitive or –resistant 30S subunits [3,26].
†IC_{50} Drug concentration that inhibits protein synthesis by 50%.

These data show that the potency of tulathromycin toward erythromycin-sensitive ribosomes is comparable to that of conventional macrolides. The data also show that tulathromycin, tilmicosin, and erythromycin are not active against erythromycin-resistant ribosomes. Newer macrolide derivatives such as the ketolide, telithromycin developed for human medicine, are generally active against resistant ribosomes. Thus tulathromycin more closely resembles erythromycin and tilmicosin rather than the ketolides or streptogramins in its activity [3,27].

Additional studies have further characterized tulathromycin binding to macrolide-sensitive ribosomes. In a competitive ribosomal binding assay measuring the displacement of ^{14}C-erythromycin from the ribosome, tulathromycin the observed equilibrium concentrations displacing 50% of the bound radiolabel (i.e., EC_{50}) for tulathromycin was 0.4 μM, compared to values of 1.5 and 0.78 μM for non-labeled erythromycin and tilmicosin, respectively) [3]. These observations suggest that the tulathromycin binding site overlaps the binding site of erythromycin.

Collectively, the results from these studies [3], support that the tulathromycin mechanism of action involves ribosomal binding, the same mechanism of action of macrolides that are commonly used in veterinary and human medicine. The inability of tulathromycin, erythromycin and tilmicosin to inhibit transcription/translation in erythromycin-resistant ribosomes distinguishes the mechanism from streptogramins and from ketolides.

1.3.2. Type of action

Macrolide blocking of protein synthesis generally has a bacteriostatic effect on the susceptible cell [28,45,46]. Macrolides are generally considered bacteriostatic agents although bactericidal effects have been observed for some drug-bacterial species combinations [28]. Ultimately this characteristic is dependent on the drug concentration, bacterial species and *in vitro* testing conditions [29,30].

MIC, MBC and time-kill kinetic studies [3] show that bactericidal activity of tulathromycin against *Campylobacter coli* and *jejuni* isolates from animals and bacteriostatic activity against animal isolates of *Enterococcus, S. aureus, E. coli* and *S. enterica* serotype Typhimurium [3]. Bactericidal activity of tulathromycin has also been observed for some of the targeted veterinary isolates [26].

1.4. Spectrum of activity

Data reviewed and accepted by the FDA/CVM show that tulathromycin is a broad-spectrum antibiotic, with *in vitro* activity against certain gram-negative and gram-positive bacterial pathogens, including the bacterial pathogens most commonly associated with bovine and swine respiratory disease [26].

1.4.1. Spectrum of activity against foodborne organisms listed in Guidance #152.

Table 2 lists the observed *in vitro* activity of tulathromycin against *Enterococcus, Campylobacter, Salmonella* and *E. coli* tested at pH 7.2-7.4, consistent with NCCLS recommended standard test conditions [31].

Table 2. Tulathromycin MICs for selected bacterial isolates.

Microorganism	No. strains	MIC (μg/ml)*		
		Range	MIC50	MIC90
Campylobacter species[†]	30	0.25 – 128	0.5	64
Enterococcus faecalis	9	4.0 - >128	8.0	NA
Enterococcus faecium	21	4.0 - > 128	8.0	>128
Enterococcus species[‡]	8	4.0 - >128	4.0	NA
E. coli	16	4.0 – 8.0	8.0	8.0
Salmonella spp.[§]	15	4.0 - >128	4.0	8.0

*Standard test conditions used consistent with NCCLS guidelines [3]. The pH of the bacteriological growth medium was adjusted to 7.2-7.4.
[†]Isolates include: 2 *C. fetus*, 13 *C. jejuni*, 15 *Campylobacter* species.
[‡]Isolates include: 1 *E. avium*, 7 *E. gallinarium.*
[§]Isolates include: 7 *Salmonella choleraesuis*, 6 *S.enterica s*erotype Dublin, 2 *S.enterica* serotype Enteritidis.

1.4.2. Effects of neutral and acidic pH on observed MIC

The *in vitro* activity of tulathromycin is dependent on the initial pH of the test medium. When the pH of the test medium was varied under standard test conditions otherwise consistent with NCCLS guidelines, the observed MICs were markedly higher at pH values at or below 7.0 compared pH range (7.2-7.4) used in MIC tests consistent with NCCLS guidance [31]. MIC results in Table 3 show that even a small shift in the initial pH of the testing medium can have a marked effect on the *in vitro* MIC values.

Table 3. Effects of pH on tulathromycin activity

Microorganism*	Mean MIC (μg/mL) at pH:†					
	6.5	**7.0**	**7.2**	**7.4**	**7.6**	**8.0**
E. coli ATCC 25922	>128	18.4	4.59	2.0	2.0	2.0
E. faecalis ATCC 29212	>128	36.8	12.1	3.48	2.0	2.30
S. aureus ATCC 29213	>128	24.3	8.00	3.03	1.74	2.0

* Quality control isolates obtained from the American Type Culture Collection (ATCC).
† Standard testing conditions consistent with NCCLS methods were used, except that pH of the culture medium was varied as indicated [31,3].

Similar pH effects were observed in two studies of *Fusobacterium* and *Bifidobacterium* reviewed and accepted by FDA/CVM. The pH in colonic contents and in feces is generally below 7.0 [32,33,34,35], although not necessarily in high forage diets in cattle [35,36].

The pH phenomenon has been documented for other macrolides [37]. It is substantial in the case of tulathromycin due to the pK_a of the three ionizable groups of the molecule [3]. Azithromycin has two ionizable groups and erythromycin has one. At neutral to acidic pH, the high positive charge of the molecule would decrease lipid solubility, concomitantly reducing the ability of tulathromycin to diffuse across cell membranes and reach the intracellular target [26,37].

1.4.3. Conclusions regarding susceptibility data

Tulathromycin has a broad spectrum of activity *in vitro*, which is substantially attenuated at a pH range normally found in the colonic contents and feces of animals.

1.5. Pharmacokinetics and pharmacodynamics

1.5.1. Absorption, distribution, metabolism, and elimination (ADME)

Extensive pharmacokinetic and disposition data have been reviewed and accepted by FDA/CVM for tulathromycin in cattle. Briefly, the pharmacokinetic profile of tulathromycin administered as a single injection at the proposed label dose is characterized by rapid and extensive absorption followed by high distribution and slow elimination [38,39]. The maximum concentration in plasma (approximately 0.5 μg/mL) is achieved approximately 30 minutes post-dosing. Peak plasma concentrations are followed by a slow decline in systemic exposure with an apparent elimination half-life of 90 hours in plasma. The bioavailability of tulathromycin after parenteral administration in cattle is approximately 90%. Plasma protein binding is low, approximately 40%. The volume of distribution at steady-state determined after intravenous administration is 11 L/kg.

Tulathromycin concentrations in lung homogenate are considerably higher than those in plasma. There is strong evidence of substantial accumulation of tulathromycin in neutrophils

and alveolar macrophages, although the *in vivo* concentration of tulathromycin at the site of pulmonary infection has not been examined.

Extensive pharmacokinetic and disposition data have been reviewed and accepted by FDA/CVM for tulathromycin in swine. Briefly, the pharmacokinetic profile of tulathromycin administered as a single injection at the proposed label dose is also characterized by rapid and extensive absorption followed by high distribution and slow elimination [39]. The maximum concentration in plasma is approximately 0.6 μg/mL, achieved approximately 30 minutes post-dosing. Peak concentrations are followed by a slow decline in systemic exposure with an apparent elimination half-life of approximately 91 hours in plasma. The observed bioavailability of tulathromycin after parenteral administration is approximately 88%. Plasma protein binding is low, approximately 40%. The volume of distribution at steady state determined after intravenous administration is 13.2 L/kg.

Tulathromycin concentrations in lung homogenate are considerably higher than those in plasma. There is strong evidence of substantial accumulation of tulathromycin in neutrophils and alveolar macrophages, although the *in vivo* concentration of tulathromycin at the site of pulmonary infection has not been examined.

1.5.1.1. Metabolism

The FDA/CVM has reviewed and accepted the metabolism files submitted by the Sponsor. These data show that tulathromycin is not extensively metabolized and is excreted in the urine and feces, mainly (>90%) as unchanged drug.

1.5.1.2. Elimination and tulathromycin residues in colonic contents post-treatment

Radiolabeled drug disposition studies in the target species have been reviewed and accepted by FDA/CVM. In studies of radiolabeled tulathromycin administered at the proposed dose to cattle and swine, approximately 30% to 60% of the total dose was excreted via the intestinal tract.

In cattle, fecal excretion and urinary excretion were comparable, each comprising approximately half of the total drug-related residues in excreta. The highest concentrations of drug residue (determined by chemical assay) in colonic contents (approximately 8 μg/g) were observed within the first day after dosing. Excreta residue levels gradually dropped and by day 5 post-dose, the concentrations in colonic contents had decreased to less than 1 μg/g [3].

For swine, approximately two-thirds of the total dose was recovered in feces, with the highest concentrations (approximately 6.0 μg/g) observed on days 3 and 4-days after dosing. Total drug residues in excreta gradually dropped and between day 6 and 12 post dose decreased to less than 1 μg/g [3].

1.5.1.3. Tulathromycin and fecal binding

Tulathromycin binds to fecal material. In equilibration fecal binding studies [3,40] wherein tulathromycin was added to a fecal slurry prepared from four bovine fecal samples, the observed adsorption coefficient (K_d) of tulathromycin was 23.3 (pH 6.7). A similar value

was obtained for a study of human fecal samples submitted to the FDA/CVM. The study showed that significant percentages of tulathromycin bound to fecal solids that readily sedimented at low speed centrifugation. Thus binding is another abiotic factor, in addition to pH (Section 1.4.2), that will attenuate the activity of tulathromycin entering the colon.

In addition to the equilibrium binding studies, the reduction in antibacterial activity of tulathromycin in the presence of fecal material was tested against various organisms. Tulathromycin activity was reduced in *in vitro* studies wherein sterilized feces were added to growth medium used for testing tulathromycin against *Enterococcus*, *E. coli*, *Bifidobacterium* and *Fusobacterium*.

1.5.1.4. Tulathromycin and pH in the colon

Studies reviewed and accepted by the FDA/CVM demonstrate a substantial attenuation of tulathromycin activity at pH $\leq$7.0, likely due to the pK_a of the three ionizable amine groups of the molecule. Whereas NCCLS guidelines specifies that the growth medium for MIC test medium is to be set between 7.2-7.4 [31], the pH of feces and colonic contents can range generally from 6.3 to 6.9 for swine and cattle (See Section 1.4.2). At these pH values, tulathromycin activity against enteric organisms is markedly attenuated (Section 1.4.2), lessening its ability to exert selective pressure for organisms *in vivo*.

1.5.1.5. Conclusions regarding drug residue in the colonic contents and feces

The tulathromycin residues in the colonic contents and feces of animals dosed parenterally is predominantly (>90%) unchanged drug [3]. Bacterial exposure to microbiologically active drug is transient due to the single dose, parenteral administration, and very low due to the abiotic factors such as pH [Section 1.4.2] and binding [Section 1.5.1.3]) that affect the ability of the drug to enter the bacterial cell upon entry of drug residue in the colon.

1.5.2. Additional effects

Post-antibiotic effect (PAE) is defined as the persistent suppression of bacterial growth *in vitro* after short exposure of bacteria to an antimicrobial agent. The effect is dependent upon the type and concentration of the antimicrobial agent, the bacterial species, duration of exposure, and experimental conditions [41]. Post-antibiotic effects have not been well-characterized, but a brief PAE would be expected after exposure to tulathromycin.

1.5.3. Pharmacodynamic properties

Although the relationship between tulathromycin and the characteristics of its antimicrobial effects have not been characterized, macrolides, as a class, tend to be primarily bacteriostatic, but may be bactericidal against some pathogens [42,43]. However, limited *in vitro* data have indicated that tulathromycin is bactericidal against some veterinary respiratory pathogens and against *Campylobacter jejuni* and *C. coli*.

Macrolides also tend to exhibit concentration-independent killing; the rate of bacterial eradication does not change once serum drug concentrations reach two to three times the MIC of the targeted pathogen. Under these conditions, the time that serum concentrations remain above the MIC becomes a major determinant of antimicrobial activity. Tulathromycin

differs from many other macrolides in that it has a long elimination half-life. This pharmacokinetic profile is well-suited to the view that macrolides are time-dependent antimicrobial agents and perform best when drug concentrations can be maintained above inhibitory concentrations for the duration of therapy.

1.6. Resistance mechanisms, genetics and location

1.6.1. Known mechanisms of resistance in animal and human pathogens

There are three basic mechanisms of macrolide resistance. Target site modification is common, and occurs either due to ribosomal RNA methylation as a result of gene acquisition (e.g., ribosomal methylases encoded by *erm*A, *erm*B, and *erm*C) or due to a sequence change in the ribosome as a result of a mutational event. The *erm* genes alter a site in the 23S ribosomal RNA needed for productive binding of macrolides, lincosamides, and streptogramin B antibiotics (MLS_B, see Section 1.8.1.1). Likewise specific mutation leading to changes in the ribosome can also impact binding [4,27,44,]. An *erm* gene has not been documented in macrolide-resistant isolates of *Campylobacter* (See Section 1.7.2.3 for further discussion). The second basic mechanism is drug inactivation, either due to phosphorylation of the 2'-hydroxy group of the amino sugar (e.g., the phosphotransferases encoded by *mph*A and *mph*B) or due to hydroxylation of the macrolide lactone ring (e.g., the lactonases encoded by *ere*A and *ere*B). The third mechanism is drug efflux (e.g., macrolide efflux pumps encoded by *msr*A and *mef*A). Acquired genes involved in resistance to macrolides have been reviewed by Roberts *et al.* [45,46] and are summarized in Table 4.

Table 4. Genes, phenotypes, and bacterial hosts of resistance determinants for the Macrolide-Lincosamide-Streptogramin group of antimicrobial agents

Resistance mechanism*	Resistance Profile*	Gene*	Host Organism(s) Described*
Ribosomal RNA methylase†	Macrolide^R, Lincosamide^R StreptograminB^R† (StreptograminA^S)	*erm*(A)	*Actinobacillus, Staphylococcus, Streptococcus*
		erm(B)	*Actinobacillus, Clostridium, Enterococcus , Escherichia, Klebsiella, Neisseria, Pediococcus, Staphylococcus, Streptococcus, Wolinella*
		erm(C)	*Actinobacillus, Bacillus, Eubacterium, Lactobacillus, Neisseria, Staphylococcus, Streptococcus, Wolinella*
		erm(D)	*Bacillus*
		erm(E)	*Streptomyces‡*
		erm(F)	*Actinobacillus, Actinomyces, Bacteroides, Clostridium, Eubacterium, Fusobacterium, Gardnerella, Haemophilus, Neisseria, Porphyromonas, Prevotella, Peptostreptococcus, Selenomonas, Streptococcus, Staphylococcus, Treponema, Veillonella, Wolinella*
		erm(G)	*Bacillus, Bacteroides*
		erm(H), (I), (N), (O), (S), (U), or (V)	*Streptomyces‡*
		erm(Q)	*Actinobacillus, Clostridium, Streptococcus, Wolinella*
		erm(R)	*Aeromicrobium*
		erm(T)	*Lactobacillus*
		erm(W)	*Micromonospora*
		erm(X)	*Corynebacterium*
		erm(Y)	*Staphylococcus*
		erm(2)	*Streptomyces‡*
		erm(30.31)	*Streptomyces‡*
Transporters	Oleandomycin^R	*ole*(B), (C)	*Streptomyces‡*
	Spiramycin^R	*srm*(B)	*Streptomyces‡*
	Tylosin^R	*tlr*(C)	*Streptomyces‡*
	Erythromycin^R	*msr*(A)	*Staphylococcus*
	Streptogramin A^R	*vga*(A), (B)	*Staphylococcus*
	Erythromycin^R	*mef*(A)	*Acinetobacter, Corynebacterium, Enterococcus , Neisseria Micrococcus, Staphylococcus, Streptococcus*
Esterases	Erythromycin^R	*ere*(A)	*Citrobacter, Enterobacter, Escherichia, Klebsiella*
	Erythromycin^R-	*ere*(B)	*Escherichia, Klebsiella, Proteus*
Phosphorylases	Macrolides^R	*mph*(A), (B)	*Escherichia coli*
	Macrolides^R	*mph*(C)	*Staphylococcus*

*Table based on Tables 2 and 3 of Reference [45] and Table 2 of Reference [46]. Even though they have chemically distinct classifications, the MLS_B agents are grouped together because they have the same mechanism of action, binding competitively to overlapping sites on the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome.

†Ribosomal methylases confer resistance to Streptogramin B components (e.g., pristinamycin IB, virginiamycin S, mikamycin B), but not to Streptogramin A components (pristinamycin IIA, virginiamycin M, mikamycin A and synergistin A) or mixtures of streptogramin components A and B (e.g., quinupristin/dalfopristin, Virginiamycin M/S).

‡These organisms are used in production of antimicrobial agents.

1.6.1.1. In vitro Induction of Macrolide Resistance

Expression (production) of *erm* genes (MLS_B phenotype) in gram-positive organisms is under either inducible or constitutive control. Some macrolides serve to stimulate inducible resistance genes, while other macrolides do not. For example, 14-membered ring macrolides such as erythromycin stimulate expression of the inducible MLS_B genes, while the 16-

membered macrolides such as tilmicosin and tylosin do not. Thus bacterial strains carrying inducible MLS_B are susceptible to 16-membered ring macrolides but are resistant to 14-membered ring macrolides. Bacteria carrying inducible MLS_B genes, however, have elevated MICs (i.e., resistance) to 16-membered ring macrolides when the expression of these genes is already induced by 14-membered ring macrolides at sub-MIC concentrations.

In studies reviewed and accepted by the FDA/CVM, tulathromycin only weakly induced a measurable degree of resistance in a *S. aureus* (pE194) strain containing a plasmid bearing an inducible *erm* gene. Thus, this compound is not a potent inducer of resistance compared with erythromycin. Tilmicosin was not active as an inducer under the test conditions, either, consistent with earlier reports [47]. The clinical significance of these observations cannot be predicted from one study. However, the three most commonly used macrolides in human health (erythromycin, azithromycin, clarithromycin) can induce resistance in gram-positive clinical isolates that possess inducible MLS_B (*erm*) genes. Historically, clinical isolates are usually inducible, while over the last decade, a significant percentage of macrolide-resistant streptococcal and staphylococcal pathogens now demonstrate constitutive *erm* expression conferring MLS_B resistance. It is possible that macrolides that are able to induce these genes and elevate the MIC may have favored the development of constitutive expression. If this is the case, then the observation that tulathromycin is a weak inducer of this gene lessen the concern for selection of constitutive resistance from those isolates containing inducible *erm* genes.

1.6.2. Location of resistance determinants

The genes encoding ribosomal methylases, macrolide transporters, and macrolide hydrolases and esterases have been documented in the chromosome and on non-chromosomal genetic elements [45,46].

1.6.3. In vitro activity of tulathromycin against human pathogens containing characterized mechanisms of macrolide resistance

Tulathromycin, erythromycin and tilmicosin were tested against a battery of clinical bacterial isolates containing known macrolide resistance genes [3]. Strains were predominantly gram-positive organisms of human origin. Genes encoding the common *ermB* determinant, as well as strains with efflux-mediated resistance to erythromycin were studied. In general, tulathromycin was less active (i.e., higher MIC's) than erythromycin against the macrolide-susceptible gram-positive organisms.

The constitutively expressed *ermB* and *ermC* resistance determinant, commonly found in clinical isolates of *Staphylococcus* and *Streptococcus*, conferred cross-resistance to erythromycin, tilmicosin and tulathromycin. However, erythromycin was inactive against the *Staphylococcus aureus* isolate containing an inducible *erm* gene, while tulathromycin and tilmicosin retained activity. The observed inactivity of tulathromycin against this strain is consistent with a weak potential to induce such genes, as observed in *Staphylococcus aureus strain* RN4220(pE194) containing a plasmid-mediated, inducible MLS_B resistance determinant (data reviewed and accepted by FDA/CVM; See Section 1.6.1.1).

Strains containing the efflux resistance determinant, *mef*A, were resistant to all three drugs, although the change in MIC was not as substantial as observed for the *erm*-containing isolates.

From these studies reviewed and accepted by FDA/CVM, tulathromycin resembles erythromycin in terms of the degree to which its activity is affected by known macrolide resistance determinants, with the exception of its comparatively weak potential to induce erm genes that are subject to induction by erythromycin. Thus, these *in vitro* studies support the premise that tulathromycin, in terms of its cross-resistance profile, has no difference in potential to select for macrolide-resistance than macrolides such as erythromycin and tilmicosin currently in use in veterinary medicine.

1.7. Occurrence and rate of transfer of resistance determinants

1.7.1. Transferable resistance determinants

The *erm* gene can be transferred among bacteria by conjugation, transposition, and by transduction [45]. Researchers have observed *erm* genes encoded on mobile elements among streptococcal isolates from animals in the early 1980's [54,55]. There are, in theory, an unlimited number of plasmid, transposon or chromosomal vectors that could serve as mechanisms for transfer. This is also the case for other macrolide resistance genes including transporters, esterases and hydrolases.

Preliminary studies reviewed and accepted by the FDA/CVM have been conducted to measure the frequency of transfer of plasmid-mediated macrolide resistance when equal concentrations of tulathromycin, erythromycin or tilmicosin are used as the selective agent [3]. The transfer system utilized the self-transmissible plasmid (pAMβ1) from *E. faecalis* JH2-2 transferred to macrolide-susceptible *E. faecalis* OG1X. The plasmid pAMβ1 [48] encodes a constitutive MLS_B gene. Plasmid transfer was detected at a high frequency of 1-2 x 10^{-2} when any of the macrolides were used for counter-selection at 50 μg/ml. The original MICs of the recipient strain to all three compounds used were 2-4 μg/ml. Therefore, an equivalent selective pressure was placed on the recipient with all three macrolides.

These limited data concerning the cell-to-cell transfer of tulathromycin resistance support that the transfer frequency of a known plasmid-encoded macrolide resistance gene is comparable whether tulathromycin or currently marketed macrolides are used in counter-selection. Since only one system generated transfer frequency data, it is not possible to make broad conclusions regarding the relative risk of resistance transfer between foodborne bacteria in the intestinal tracts of livestock or humans. It is not clear how many mating pairs and plasmid types (or what experimental conditions for testing) would be needed to predict selection pressure *in vivo.* However, given the similar mechanism of action and spectrum of tulathromycin compared with other macrolides, it is expected that the selective pressure for resistance transfer will be similar for macrolides already used in poultry, swine, and cattle. Additionally, at the neutral to acidic pH of the colonic contents and feces, the microbiological activity is attenuated.

1.7.1.1. Campylobacter

In molecular examination of macrolide-resistant isolates, the resistance acquisition is demonstrated to be due to point mutations in the chromosome of *Campylobacter* (Section 1.7.2.3). Thus studies of transfer rates of this gene are not applicable to this organism. Transferable elements encoding macrolide resistance have not been documented.

1.7.2. Point mutations

1.7.2.1. Characterization of field isolates for studies of point mutation frequencies

A collection of 57 isolates of *E. coli, Salmonella, Enterococcus,* and *Campylobacter* were used in pure culture studies to estimate the rates of mutation to macrolide resistance. The isolates were screened for presence eight common macrolide resistance determinants (*erm*A, *erm*B[1], *erm*C, *ere*A, *mph*A, *mph*B, *msr*A and *mef*A) by molecular and phenotypic methods. The analyses supported that there was an absence of inducible macrolide resistance genes (known or uncharacterized) in the strains tested [3]. Thus this collection of isolates was deemed suitable for evaluating mutation frequencies *in vitro* (Section 1.7.2.2).

1.7.2.2. Mutation frequencies

The FDA/CVM has reviewed and accepted a study submitted by the Sponsor wherein the frequency of mutation of *E. coli, Salmonella, Enterococcus* and *Campylobacter* were determined *in vitro* by recognized microbiological methods. The collections of isolates described above were plated in pure cultures on medium containing inhibitory concentrations of tulathromycin to select for growth of spontaneous mutants resistant to the triamilide. Resistant mutants were not detected. Since at least 10^8 to 10^9 colony-forming units (CFU) of each susceptible strain were plated, the frequency of spontaneous mutants resistant to tulathromycin was less than 10^{-8} or less than 10^{-9}.

These results suggest that the frequency of mutation to tulathromycin occurs *in vitro* are very low, and are consistent with low frequency of occurrence of spontaneous mutations in ribosomal genes observed with other macrolides [4,1].

1.7.2.3. Campylobacter

The frequency of resistant mutants in *C. jejuni/C. coli* strains exposed to concentrations of tulathromycin equal to 4- to 8-times their MIC was the mutation rate expected for spontaneous mutation (Section 1.7.2.2).

To date, all macrolide resistance has been documented to occur by mutation in the chromosome encoding ribosomal RNA of *Campylobacter* [49,50,51]. There are a few reports of macrolide-resistance due to mutation in efflux pumps in *Campylobacter* (Section 1.8.1.3). Jensen and Aarestrup [49] described a collection of 54 cattle and swine isolates of *C. coli* that were resistant to erythromycin (MIC > 8 μg/ml). All of the resistant strains contained a mutation in position 2230 of the 23S ribosomal DNA, a change consistent with

[1] The PCR amplification conditions for *ere*B (enzyme hydrolyzes macrolide lactone ring) could not be established since the *ere*B primer did not show consistent results against positive and negative control DNAs.

spontaneous mutation. These results are consistent with the low frequency of mutation observed to tulathromycin in the studies described in Section 1.7.2.2. Spontaneous mutation in chromosomally encoded ribosomal genes is the most likely mechanism by which clinically relevant macrolide resistance occurs in *Campylobacter*. This is consistent with the observed rates of low macrolide resistance in essential genes *Campylobacter*.

1.8. Resistance selection pressures

1.8.1. Information regarding cross-resistance to other antimicrobial drugs approved in veterinary and human medicine

All phenotypic, biochemical, and molecular studies support that tulathromycin, erythromycin and tilmicosin have similar profiles with respect to known resistance determinants of *Enterococcus, Streptococcus* and *Campylobacter,* with the exception of *erm* genes that are inducible. Unlike the human use macrolides (erythromycin, clarithromycin and azithromycin), both tilmicosin and tulathromycin (as well as the lincosamides and the streptogramins) are poor inducers of expression of inducible *erm* genes. Cells harboring constitutively expressed *erm* genes are cross-resistant to the entire MLS_B group of molecules ([45,46] see Section 1.8.1.1).

Macrolides have been used extensively in human and animal medicine for years (see Section 1.8.2.2). Rates of macrolide resistance in *Enterococcus* have been high since the 1970's in the United States [2]. Therefore, the impacts of tulathromycin use in view of the widespead and prolonged macrolide use can be very difficult, if not impossible, to project because selective pressure has already occurred and continues due to other macrolide use practices.

1.8.1.1. Macrolide-Lincosamide-Streptogramin B (MLS_B) group classification and cross resistance

Even though they have chemically distinct classifications[2], the MLS_B agents are grouped together because they have the same mechanism of action, binding competitively to overlapping sites on the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome [25,28,52,53]. Cross-resistance to MLS_B (but not streptogramin component A) can occur due to mutation or due to acquisition of an *erm* gene resulting in changes in the 23S ribosomal RNA. Note that other types of acquired resistance mechanisms (e.g., change in cell permeability, drug inactivation) do not confer complete cross-resistance among the macrolides, lincosamides and streptogramins [45,46]. Constitutively expressed *erm* genes are the only genes that confer complete cross-resistance to the MLS_B group (but not streptogramin mixtures). *Erm* genes, notably *erm*B, have been demonstrated in enterococci, but, to date, *erm* genes have not been demonstrated in *Campylobacter*.

[2] Macrolides are macrocycline lactone peptolides. Macrolides can be divided into 14-membered (erythromycin, clarithromycin), 15-membered (azithromycin, tulathromycin), and 16-membered (tylosin, spiramycin) lactone ring macrolides. Commercially available streptogramins are cyclic peptides, and comprised of an "A component" and a "B component" which act synergistically. The A components are polyunsaturated macrolactones, consisting of lactam and lactone linkages with an oxazole ring. The B components are cyclic hexadepsipeptides. The lincosamides consist of an amino acid linked to an amino sugar, and are devoid of a lactone ring.

1.8.1.2. MLS_B Resistance in Gram-positive cocci from humans

Macrolide resistance due to an *erm* gene has been documented since the 1950's among human isolates, soon after the introduction of erythromycin for use [45,28]. At first the resistance was characterized as being inducible by erythromycin (and other 14-membered ring macrolides), but constitutive resistance conferring cross-resistance among the MLS_B components was also detected.

Rollins *et al*. [54] documented macrolide resistance and multi-resistant streptococcal isolates in swine, chickens and humans from collections obtained from human patients, and healthy animals in 1979 and 1980. Subsequent studies by LeBlanc *et al*. in 1986 [55] showed that selected isolates of Group D streptococci contained DNA that was consistent with sequence homology for resistance to erythromycin, kanamycin and streptomycin. These studies at minimum demonstrate that *erm* genes have been broadly disseminated since as early as the 1980's if not before this time point in the US.

Given that the dissemination was already high 20 years ago, it is difficult to dissect the relative contribution of any antimicrobial use pattern, including proposed tulathromycin use, to influence the pattern of such resistance determinants in commensal or pathogenic organisms of man or animals.

1.8.1.3. Campylobacter

Campylobacter isolates that are sensitive to erythromycin are also sensitive to tulathromycin under standard test conditions. Tulathromycin MICs for macrolide-sensitive *Campylobacter* species in a sample of 30 strains ranged from 0.25 to 1 μg/ml [3]. *Campylobacter* resistant to erythromycin had high MICs to tulathromycin (32 to 128 μg/g). This cross-resistance with other macrolides is consistent with the mechanism of action of tulathromycin of binding to the ribosome (See Section 1.3).

In addition, an efflux pump found recently in *Campylobacter* isolates confers elevated MICs not only to macrolides, but also to other antimicrobial agents, including tetracyclines, fluoroquinolones, to other antimicrobial classes, as well as other types of compounds, such as dyes, etc. [56,57, 58] Therefore it is feasible that a number of different antimicrobial agents and other types of compounds used in animal medicine may exert selective pressure for *Campylobacter* having higher macrolide MICs.

1.8.2. Information regarding co-resistance to other antimicrobial drugs approved in veterinary and human medicine

1.8.2.1. Consideration of the extent of use of the proposed product

1.8.2.1.1. Extent of use of tulathromycin

Tulathromycin has a pharmacokinetic profile that produces a rapid onset of action, high concentrations of active drug in target tissues and a long duration of action [3].

- **Duration of administration - Tulathromycin** is administered as a single injection for the proposed disease indications on the label.

- **Individual vs. small groups vs. flocks/ herds -** As an injectable, tulathromycin is an individual animal treatment. It is administered to cattle with clinical signs related to BRD and to animals identified to be at high risk of developing BRD. In swine, it is administered to animals with signs of SRD.
- **Dosage -** The FDA/CVM has reviewed and accepted data regarding the efficacy of tulathromycin administered at the proposed dosage in a single dose.
- **Treatment period -** Tulathromycin is to be administered as a single injection treatment for the disease indications on the label.
- **Use pattern -** Tulathromycin provides an alternative for or replaces existing treatments. Its use is not expected to expand the use of antimicrobial agents for animals suffering from BRD or SRD. This product will not change the incidence of BRD, nor will it change the criteria feedlot managers and veterinary consultants use to determine if arriving cattle are at risk of developing BRD. The proposed uses of tulathromycin are consistent with judicious use principles outlined by the FDA/CVM in collaboration with the American Veterinary Medical Association [59],60].

Tulathromycin provides a complete course of treatment with a single injection in both cattle and swine. This is particularly important since handling associated with multiple injections increases stress on the animal and increases costs associated with disease and disease therapy. The pharmacokinetic/pharmacodynamic profile of tulathromycin enables this single injection treatment regime, assuring compliance for a full course of therapy. Approval also increases the range of products from which to choose. Approval of tulathromycin represents a major advance in the treatment of livestock respiratory disease.

1.8.2.2. Extent of use of macrolides in cattle and swine

The macrolides tylosin and erythromycin are available for use in swine in oral and parenteral formulations, and tilmicosin is also available as an oral formulation for swine. Historically, tylosin is the most commonly used macrolide agent in swine for a wide variety of indications in including pneumonia, erysipelas, weight gain/feed efficiency, controlling swine dysentery and *Mycoplasma* arthritis. Tylosin has been approved for use since 1961. Tylosin also is approved for a number of poultry indications. Tilmicosin has been one of the most widely used injectable antimicrobials in cattle for the treatment of BRD and for the treatment of cattle at high risk of BRD following its approval in 1992.

Frequently organisms carrying an *erm* gene are multi-resistant to other drug classes in addition to the MLS_B group [54,55,61,62,63] and therefore the use of a number of antimicrobial agents may exert selective pressure for an *erm* resistance determinant. However, the existing, widespread use of macrolides in cattle and swine is detailed below.

1.8.2.2.1. Extent of use of macrolides in cattle

The United States Department of Agriculture/National Animal Health Monitoring System (USDA NAHMS) conducted a survey of beef feedlot production sites in 1999 in 12 states representing 84% of the US feedlot inventory [64]. Approximately 42% of all feedlot cattle

received tylosin orally in the feed or water as a health or production tool, with cattle remaining on the product for an average of 138-145 days, depending on arrival body weight. In the same survey, injectable macrolides were the primary treatment in over two-thirds of the cattle that received antimicrobial treatment upon arrival, and injectable macrolides were the primary initial treatment for nearly one-third of cattle requiring treatment for BRD.

The same USDA NAHMS survey of 1999 [64] indicated that BRD is the overwhelmingly predominant disease condition in cattle arriving at the feedlot, with nearly 15% of the cattle showing signs of BRD after arrival, currently estimated at 25-27 million cattle. Vogel [65] estimated mortality attributable to BRD at between 65 and 79% of all types of death in the feedlot. Loneragan *et al.* [66] estimated that respiratory disease counted for 57.1% of all feedlot deaths, accounting for approximately 7 deaths per 100 cattle (99.8%) in feedlots. In addition, the 1999 USDA NAHMS survey [67] indicated that 19% of all cattle received to a feedlot receive an injectable antibiotic, and that approximately 10.4% of all arriving cattle are treated on arrival with some antibiotic [64]. Nearly all feedlots surveyed used some form of antimicrobial as treatment for BRD, with feedlots having more than one antimicrobial as potential initial treatment. Over 31% of surveyed feedlots used tilmicosin as the primary initial treatment for BRD; florfenicol, tetracyclines, and ceftiofur were used as the primary initial treatment in 21.9%, 21.6%, and 6% of feedlots, respectively [64].

1.8.2.2.2. Extent of use of macrolides in swine

The USDA NAHMS conducted a survey of swine production sites in 17 states representing 94 percent of the US pig inventory and 92 percent of US pork producers with 100 or more swine during the survey period Dec 31, 2000-May 31, 2001. Among sites housing nursery pigs, 82.7% of the sites fed antibiotics for growth promotion or disease prevention [68,69]. Among these sites, 37.8% reported feeding a macrolide [68,69]. Macrolide (tylosin) use among grower finisher sites was reportedly higher in the survey: 56.3% of the sites reported used in feed, 30.7% of the sites by injection, and 4% of the sites in water.

Respiratory disease was ranked as the number one producer-identified cause of mortality in both nursery pigs and grower/finisher swine, responsible for 28 percent of nursery deaths and 40 percent of grower/finisher mortality [70]. In addition, two separate bacterial respiratory diseases (*Mycoplasma* pneumonia and *Actinobacillus pleuropneumoniae*) were ranked in the top ten disease problems in grower/finisher swine. Injectable antibiotics represent a fraction of the overall therapeutic antibiotic use in swine, according to the Doane Animal Health Marketing Survey data (provided by Doane to the sponsor). As such, injectable penicillin commands nearly two-thirds of the market share (in terms of doses), with injectable oxytetracycline, tylosin, lincomycin, and ceftiofur also being used. In NAHMS studies [71], one third of surveyed swine production sites used antimicrobials in grower/finisher swine, primarily to treat/control respiratory disease. Although entire pens are treated, the duration of therapy was approximately 4-6 days. Approximately 27% of production sites used antibiotics in feed to treat respiratory disease, compared with nearly 38% that use antibiotics in feed that use antibiotics in feed for disease prevention. The duration of treatment in fed for treatment f respiratory disease ranged from 15-39 days.

1.8.3. Conclusions regarding selection pressure

The impact of tulathromycin use on *Campylobacter* is expected to be minor due to the abiotic factors in the colonic content and in feces (i.e., pH, binding), which attenuate the microbiological activity by pH. Given the similarities in mechanism and resistance profiles with macrolides used in animal medicine for therapeutic and growth promotion uses, and the fact that tulathromycin is to be administered once as a single dose regimen, parenterally by prescription, only to individuals under veterinary prescription, there is no expectation that tulathromycin use will add significant additional selection pressure for resistance emergence in campylobacter, or other organisms.

Macrolides have been and are available for use in animals in injectable, oral and topical formulations. They have been approved for more than 30 years for therapeutic, metaphylactic and growth promotant indications in poultry, swine and cattle. However the observed rate of macrolide resistance in *Campylobacter jejuni* isolated from humans is 1-3% since 1989, with no trends over time (Table 5). Mobile genetic elements encoding macrolide resistance have not been reported in *Campylobacter*. Therefore, given this historical perspective and experimental data, it is reasonable to propose that macrolide use in general and tulathromycin use specifically for therapeutic use in cattle and swine production at times of respiratory infections are not expected to have a significant impact on release of macrolide-resistant organisms. The experimental evidence that tulathromycin does not select for unique macrolide resistance determinants in *Campylobacter* shows that tulathromycin has no higher likelihood for selection of resistant organisms than macrolides already in use.

1.9. Baseline prevalence of resistance

Campylobacter, including macrolide-resistant strains, are found in cattle and swine on the farm and at slaughter. However as discussed fully in Section 2, contamination of beef and pork products with *Campylobacter*, either macrolide-sensitive or macrolide-resistant, is infrequent.

The NARMS surveillance reports for *Campylobacter* isolated from animals are collections of poultry isolates [72]. However, US national surveys of macrolide resistance among *Campylobacter* isolates in cattle and swine are lacking. This lack of emphasis on *Campylobacter* contamination from beef and pork may be due in part to epidemiological reports suggesting that risk factors for campylobacteriosis due to beef or pork is low [104, 112, 113, 114], compared to risk factors of consumption of poultry or, to a lesser extent, raw milk.[3]

In examining the resistance surveillance programs for resistance in *Campylobacter*, it is important to recognize that 1) various programs have used different sampling strategies and isolation procedures, which may or may not include use of antimicrobial agents for isolation of *Campylobacter* from animal or meat sources; 2) NCCLS performance standards for susceptibility testing of *Campylobacter* have only recently issued in 2003; and testing can

[3] Tulathromycin is not intended for use in lactating dairy cattle.

vary among various surveys due to differences in test systems; 3) NCCLS has not established macrolide breakpoints for *Campylobacter*, and the macrolide breakpoints and test media for other organisms (e.g., anaerobes vs. aerobic cocci) are substantially different [8].

The NARMS program uses and E-test to monitor drug MICs of *Campylobacter* isolates from humans [6]. Although performance standards are not published by the NCCLS, the E-test method used is consistent across years in the NARMS program, permitting comparisons of MICs from one year to the next.

1.9.1. NARMS MIC data for Campylobacter

The United States NARMS program includes surveillance of *Campylobacter* for humans and for animals. Table 5 summarizes MIC data for *Campylobacter* isolated from humans in the United States under this program [6,73,93]. *Campylobacter* isolates show 1-2% prevalence of macrolide resistance (erythromycin MIC$\geq$ 8 μg/mL by E-test methods) among *C. jejuni* isolates (n= 209-365 isolates/year). These rates were comparable to an earlier 1992 survey of roughly 332 isolates tested by E-test [73]. The numbers of isolates from *C. coli* from humans are few, making the analysis of trends over time difficult; however, the prevalence of macrolide-resistance in *C. coli* is numerically higher for than for *C. jejuni*, which has been similarly documented worldwide [51].

Table 5. MICs of macrolides tested against *Campylobacter*

Ref	Source	Test method	Location	Species	Survey date	N	Erythromycin MIC (μg/mL)*				Azithromycin MIC (μg/mL)*			
							Range	MIC_{50}	MIC_{90}	%R*	Range	MIC_{50}	MIC_{90}	%R**
93	Human	microbroth dilution	19 sentinel county labs	*C. jejuni*	1989-1990	286	NR	NR	NR	1%	NR	NR	NR	1%
73,93	Human	E-test	CDC sentinel sites	*C. jejuni, coli*	1992	332 (preliminary)	NR	NR	NR	2%‡	NR	NR	NR	NR
6,93	Humans Human	E-test	CDC sentinel sites	*C. jejuni*	2001	365	≤0.38-≥48	0.75	1.5	2%	≤0.064-≥8	0.125	0.25	2%
					2000	306	≤0.38-≥48	0.75	1.5	1%	≤0.064-≥8	0.125	0.25	2%
					1999	294	≤0.38-≥48	0.75	1.5	2%	≤0.064-≥8	0.125	0.375	3%
					1998	297	≤0.38-≥48	0.75	2.25	2%	≤0.064-≥8	0.188	0.375	1%
					1997	209	≤0.38-≥48	1.0	3	1%	≤0.064-≥8			
				C. coli	2001	17	≤0.38-≥48	0.75	4.5	6%	≤0.064-≥8	0.375	0.75	6%
					2000	12	≤0.38-≥48	1.0	4.5	8%	≤0.064-≥8	0.375	1	8%
					1999	20	≤0.38-≥48	1.0	3	10%	≤0.064-≥8	0.188	0.75	10%
					1998	8	≤0.38-≥48	NA	NA	12%	≤0.064-≥8	NA	NA	12%
					1997	6	≤0.38-≥48	NA	NA	0%	≤0.064-≥8	NA	NA	0%

*Erythromycin resistance defined as MIC ≥8;

** Azitrhomycin Resistance defined as MIC ≥ 2 μg/mL

Currently, the NARMS program for antimicrobial susceptibility testing of *Campylobacter* isolates from animals only monitors poultry isolates [6]. The erythromycin resistance (MIC ≥8μg/mL) rates for poultry isolates of *C. jejuni* (n=128-590 isolates/year) ranged from 0.2-5.1% for years 1998-2003 with no obvious trend over time [74]. The erythromycin resistance (MIC ≥8μg/mL) rates for poultry isolates of *C. coli* (n=63-288 isolates/year) ranged from 11-23% for years 1998-2003 with no obvious trend [74].

The NARMS surveillance program does not currently monitor susceptibility profiles of *Campylobacter* isolated from cattle or swine [72,74]. A literature search did not reveal published US national surveys of antimicrobial susceptibility profiles of *Campylobacter* species isolated from swine. However, Englen *et al.* [75] reported a survey of antimicrobial sensitivity of *Campylobacter* isolated from healthy feedlot cattle in the US. Isolates were obtained from feedlot cattle as part of the 1999 USDA NAHMS monitoring survey of health feedlot cattle [64,75]. Fecal samples were collected from cattle by state veterinarians at feedlots in states representing 90% of the cattle fed in the US. The prevalence of macrolide resistance (MIC ≥ 8 by E-test method), was 2.2% of the *C. jejuni* (n=92) and 7.7% (n=26) of *C. coli* isolates obtained in the study. The report did not provide information regarding the total number of feedlots or cattle sampled to obtain the 118 isolates in the study. Among the four total isolates of the 118 isolate collection that were resistant to erythromycin, two *C. jejuni* and one *C. coli* isolates were multi-resistant to at least three other drug classes.

Ge *et al.* reported the MIC values of *Campylobacter* isolated from retail meats collected in Washington, DC [76], showing 17% erythromycin resistance among isolates from poultry. Of other meat samples analyzed for *Campylobacter* in the same study, only three of 181 pork samples, and 1 of 182 retail beef samples were positive [77] for this organism. The authors did not report on the MICs of the four pork and beef isolates in the collection. White *et al.* presented a preliminary report on the macrolide resistance among *Campylobacter* isolates from the FDA/CVM Retail Meat Sentinel Site Survey [7]. Results of surveys and reports from the EU are consistent with data on *Campylobacter* susceptibility patterns in the US, as surveyed in Section 1.9.2.

1.9.2. Other MIC surveys of Campylobacter isolates

1.9.2.1. Isolates from humans

There are few published surveys of macrolide resistance in *Campylobacter* in the United States besides those reported via the NARMS program [6]. Nachamkin *et al.* [78] reported 2% overall erythromycin resistance among 142 isolates from patients from Pennsylvania collected 1982-92, and fluctuated from 0-5% among 297 patients 1995-2001, with 3.5% recorded in 2001 (E-test method, [79]).

In Canada, Gaudreau and Gilbert reported 0% erythromycin resistance (MIC ≥ 8 μg/mL tested by agar dilution) for 291 isolates collected from patients in Montreal, Quebec 1985-6, 1992-3 and 1995-6 [80]. In a later report by the same authors, yearly resistance rates varied from 1 to 12% in 1998-2001 (51-72 isolates/year), with no statistically significant trend detected [81]. These reports are consistent with the rate reported for Canada earlier by

Karamali, where erythromycin resistance (MIC $\geq$ 8 μg/mL by agar dilution) was 0.5% among *C. jejuni* tested isolated from Canada before 1981 [82].

Wagner *et al*. [83] recorded macrolide resistance among *C. jejuni* isolates from Germany from 1998-2001, using a standard method. The prevalence rate of erythromycin resistance (MIC $\geq$ 8 μg/mL) was 4.2% (n=144). Luber *et al*. [84] recorded micro-broth dilution results of 1.5% (n=68) and 0% (n=65) erythromycin resistance (MIC $\geq$ 8 μg/mL) in 1991 and 2001, respectively, from *C. jejuni* isolates from patients in Berlin. *C. coli* isolates were higher, with 7.1% (n=14) and 29.4% (n=17) resistance to erythromycin in 1991 and 2001, respectively. Krausse & Ullmann [85] reported yearly results of *C. jejuni* and *C. coli* isolates; erythromycin MIC values $\geq$ 8 μg/mL fluctuated between 0-2% in survey periods 1980-82 (n=30), 1997-8 (n=93), 1999-2000 (n=85) and 2001 (n=99) in Germany.

1.9.2.2. Isolates from animals

Van Looveren *et al*. [86] reported that 67% of the 61 isolates of *C. coli* from swine in Belgian slaughterhouses in 1998 were erythromycin resistant (MIC $\geq$8 μg/mL, tested by agar dilution). Macrolide resistance rates of *C. jejuni* isolates from poultry (n= 285) were 6.3% and 8.6% in broilers and layers, respectively. The macrolide MIC values of the four swine isolates of *C. jejuni* were not reported.

1.9.2.3. Macrolide resistance in C. jejuni vs. C. coli

The higher prevalence rates of macrolide resistance of *C. coli* compared to *C. jejuni* have been recognized since *Campylobacter* susceptibility reports first began in the late 70's and early 80's, soon after methods were developed for routine culture of this fastidious genus in the laboratories worldwide [51,87,88,82,89,90,91,9293]. Engberg *et al*. [51] provided a listing of surveys reported since 1989 of azithromycin and erythromycin resistance in *Campylobacter* isolates from humans. Although the reported resistance rates ranged from 0-11% for *C. jejuni*, and from 0-68% for *C. coli*, no conclusions can be drawn regarding trends or baseline prevalence, since the reports used different sampling methods, sensitivity test methods, and criteria for scoring resistance. The authors noted trends for fluoroquinolones, but did not draw conclusions for trends in macrolides. The reasons for the differences in prevalence of macrolide resistance among *Campylobacter* species is not clear. Macrolide resistance is higher in *C. coli* isolates from poultry than *C. jejuni* in poultry (NARMS report 2003[94]). Studies are needed to better understand this observation.

1.9.3. Conclusions regarding baseline MIC data for Campylobacter

The prevalence rates of macrolide resistance are between 1-3% among all human isolates of *Campylobacter* in the NARMS program 1997-2001, with no apparent trends over time. The macrolide resistance rates of *C. jejuni* vary similarly (1-3%; 209-365 isolates/year). The rates for *C. coli* from humans are more difficult to estimate (0-12%, n= 6-20 isolates/year) than *C. jejuni* rates because the frequency of *C. coli* isolation is so low among humans in the United States (as observed in many other countries). *C. jejuni* isolates outnumber *C. coli* isolates by more than 10-fold in the NARMS program for surveillance of human *Campylobacter* isolates. Nonetheless, overall macrolide resistance rates are low despite

decades of macrolide and lincosamide use humans, swine and other food-producing animal species in the United States.

Prevalence data are insufficient in the United States to determine national rates of resistance among *Campylobacter* isolates from swine and cattle. No United States national surveys have been published for isolates from swine. The 1999 survey of feedlot cattle [75] yielded an overall rate of 3% erythromycin resistance among *Campylobacter* isolates.

Macrolide resistance has been observed to often be >20% among most surveys of *C. coli* whether isolated from swine or other animal species in studies conducted in other parts of the world, and in all reports of *C. coli* conducted since methods were developed to culture these organisms in the late 1970's. However, *C. coli* represent a minority of cases causing campylobacteriosis in surveys of human clinical isolates in the United States and elsewhere in the world.

1.10. Other relevant data

In an early exploratory study[4] the potential for tulathromycin to eliminate the carrier state of *Salmonella typhimurium* in growing swine was evaluated. Swine were challenged with a strain of *S. typhimurium* with a tulathromycin MIC of 1.56 μg/ml and were later dosed once intramuscularly with tulathromycin at either 10 or 15 mg/kg body weight (10 pigs/group); a control group received a single IM dose of saline. Subsequent to treatment, fecal samples were collected from pigs for *Salmonella* analyses through 28 days post-treatment. Based on the quantities of *Salmonella* shed and the proportion of pigs shedding any salmonellae, it was concluded that *Salmonella* shedding in the groups of pigs treated with either dosage of tulathromycin were similar to the control pigs. Separate excretion studies with tulathromycin in pigs showed residues in feces of 10 to 70 μg/g in the first three days following an IM dose of 2.5 mg/kg; thus exposure of salmonellae to residues following dosing regimen of 10 and 15 mg/kg in the exploratory study would be expected to be substantially larger. Despite a challenge strain MIC of 1.56 μg/ml and the relatively high expected drug exposure, salmonellae populations were apparently not impacted by the tulathromycin treatments. This observation is consistent with an expected lack of bioactivity of drug residues in the colon, even in the absence of passage through the stomach.

This study does not address the resistance selection *per se*. However, this study supports the expectation that the tulathromycin MICs determined for pure cultures of enteric bacteria under standard test conditions does not predict the potency of this drug *in vivo* in the colon contents or in feces. Other factors not taken into account in the MIC test, such as neutral to acidic pH and binding to fecal particles are expected to attenuate the activity of tulathromycin [Sections 1.4,1.5.1.3]. The observation that S*almonella* shedding in this experimental swine model was not appreciably affected *in vivo* when tulathromycin was administered supports that tulathromycin microbiological activity in the colonic contents and feces is highly attenuated, lessening the risk for resistance selection in the gut.

[4] The study report is submitted with this filing as supportive data for reference; since it was part of an drug discovery program, the study has not previously been submitted.

1.11. Overall Release Assessment Conclusion: "Low"

The following table summarizes the components of the release assessment requested in Guidance #152.

Relevant parameters	Extent to which relevant factors favor resistance emergence
	Comments/conclusions regarding factors
Mechanism of activity	Inhibits protein synthesis by binding to the bacterial ribosome. Bacteriostic or bactericidal. Bactericidal against *Campylobacter*
Spectrum of activity	Broad spectrum .
Pharmacokinetics	-Rapid,extensive absorption, -High volume of distribution,slow elimination. -Plasma protein binding is low (40%) -Maximum plasma concentration 30 min after dose -Bioavailability is high (88-90%)
Pharmacodynamics	-Macrolides may exhibit post-antibiotic effects. Thus the potential exists for continued activity for a short period after the drug is removed.
Resistance mechanisms	-3 mechanisms: 1) target (ribosomal RNA binding site) modification, 2) drug efflux, 3) drug inactivation. -Chromosomal mutation of ribosomal DNA is most relevant to macrolide resistance in *Campylobacter.*
Resistance transfer	-Resistance transfer important for enterococci, streptococci, many organisms -No reports to date of macrolide-resistance transfer in *Campylobacter* (all macrolide-resistance characterized to date due to mutation)
Selection pressure	-Total residue (microbiologically active and inactive) in colonic content and feces is attenuated due to abiotic factors: 1) neutral to acidic pH of colonic contents and feces 2) binding to fecal substrates. - Macrolides have been approved and in use in cattle, swine, poultry for over 30 years. -Tulathromycin is administered to individual animals -Tulathromycin is to be administered by veterinary perscription only -The single-dose regimen enhances the potential for user compliance related to full course treatment, and reduces animal stress associated with repeated dosing at the animal production site. -Alternative therapies (including other macrolides) are available. Tulathromycin use is expected to displace existing macrolide use, and not increase macrolide or antibiotic use.
Baseline prevalence of resistance	-*C. jejuni* resistance to macrolides is $\leq$3% among human isolates tested *in vitro*, with no trends over time during NARMS surveillance. -*C. coli* isolates from swine have higher percentages of macrolide resistance, which has been documented worldwide since the time *Campylobacter* was first cultivated and tested. -*C. coli* represents <10 % of isolates from humans (NARMS data)
Other factors	-Respiratory diseases requiring treatment generally occurs during early stage of production, long before animals are ready for entry into the food chain.

Campylobacter jejuni and *C. coli* is found in cattle and swine as part of the intestinal flora and will be exposed to tulathromycin where therapy is needed. Selection of macrolide resistance by spontaneous mutation would occur at a very low frequency. These mutational

events are deemed of low significance, as evidenced by the long use of macrolides in animals, and low prevalence rates of macrolide resistance in *Campylobacter* in humans.

Macrolide resistance has not been demonstrated to be transferable in *Campylobacter*. After more than three decades of macrolide use in animals and in humans, the rates of macrolide resistance in *Campylobacter* isolated from humans remains low in the US. Thus macrolide use in livestock, companion animals and in man has not had a significant impact on the level of pre-existing macrolide resistance in *Campylobacter*.

The overall conclusion is that there is a “Low” probability of release of macrolide-resistant *Campylobacter* as a result of tulathromycin use.

2. EXPOSURE ASSESSMENT

2.1. Overview

Table 5 of FDA/CVM Guidance #152 [1] (Figure 1), provides a possible process for ranking qualitatively the probability of human exposure to a given bacteria in food commodities based on national surveys of food commodity consumption in the United States and food commodity contamination rate data. The algorithm used to rank overall exposure, based on these rates is copied in Figure 1.

Using the default values provided in Guidance #152 for beef (consumption rate of "High" and *Campylobacter* contamination rate of "Low"), the overall exposure assessment for beef is "Medium", based on this algorithm.

Using the default values for pork (consumption rate of "High" and C*ampylobacter* contamination carcass contamination rate of "High", the overall exposure assessment for pork is "High". However, *Campylobacter* epidemiological evidence, *Campylobacter* contamination rate data in carcass and at retail, and processing in pork shows that the contamination rate of retail pork meat is consistently low (contamination rates in retail pork <5% [Section 2.4.3.3]). By applying the "Low" contamination rate categorization found in Guidance #152 and using its algorithm for ranking the probability of human exposure, the overall exposure assessment for pork is categorized as "Medium".

These qualitative exposure assessments of "Medium" for human exposure to *Campylobacter* via beef or pork are conservatively high estimates. Epidemiological studies indicate the primary risk factors for campylobacteriosis in humans, for both outbreaks and sporadic cases are consumption of raw milk/milk products and untreated surface water, as well as handling and consumption of raw or undercooked poultry. The observed frequency of isolation rates from beef and pork meats at retail are consistently low.

2.2. Campylobacteriosis in man and its epidemiology

The principle reservoir for *Campylobacter* is the alimentary tract of wild and domesticated animals and birds. In the NARMS program from 1997-2001 for monitoring *Campylobacter* isolated from humans, from 8 to 20 *C. coli* isolates were profiled per year, compared to 209-

365 *C. jejuni* isolates are per year [73]. This ratio of species in humans is consistent with surveys of *Campylobacter* in humans worldwide. *C. jejuni* is most frequently isolated in cases of campylobacteriosis in humans and estimated to be the predominant species responsible *Campylobacter* gastroenteritis [95].

C. jejuni is predominantly associated with poultry and cattle, whereas *C. coli* is predominantly associated with swine [101]. Ingestion or preparation of poultry or poultry products, non-pasteurized milk or milk products, contaminated water or zoonotic exposure are frequently listed among the highest risk factors generally for campylobacteriosis due to *C. jejuni* [96]. Risks for sporadic cases, accounting for the majority of cases, also include handling/consumption of raw or undercooked poultry as a primary factor. Poultry products have generally been implicated as the primary vehicle for transmission of foodborne *Campylobacter* to humans [96,97,98].

Although *C. coli* is prevalent on surface samples of swine carcasses [102,99,100], it is apparent that the contamination rates of *Campylobacter* are substantially lower during pork meat processing and at retail (see Section 2). Epidemiological studies support this observation. There are very few documented cases of human infection by *Campylobacter* from pork products and an abundance of documented cases of infection from poultry products and, and to a lesser extent, raw milk [97,101]. *C. jejuni* is the species most frequently isolated from poultry products [77,92, 97, 101]. In a summary of outbreaks with a known etiology involving pork between 1990-1997 in the USA, only 2% of foodborne illness due to pork had an etiology associated with *Campylobacter* [102]. Frequently pork consumption is not mentioned as a risk factor for *Campylobacter* disease in humans [96]. In a recent study examining the risk of transmission of *Campylobacter* from swine to man, the author concluded that transmission of *C. jejuni* was non-evident, and pork consumption was a very low risk for *C. coli* [92].

Gillespie *et al.* [103] used a case-case analysis to compare *C. coli* infections with cases of *C. jejuni* infection to generate hypotheses for infection based on sentinel surveillance information in England and Wales. They postulated risk factors for *C. coli* to be consumption of bottled water, ingestion of pâté (frequently pork liver in the UK) or meat pies (meat source unknown), based on this analysis. Neither pâté nor meat pies are commonly prepared from pork in the United States diet.

Recent molecular analysis of *C. coli* isolates from swine and poultry suggest that host specificity of strains of *C. coli*, which may in the future help to better understand the epidemiology of *C. coli* [104]. A Swedish case control study identified an odds ratio for "bone-in" pork, but the authors cautioned that further study is needed to confirm this statistic [105]. In a report by the CDC in 1992 on the epidemiology of *C. jejuni*, pork products were not listed as a risk factor, with poultry, eggs, raw milk, poultry, and drinking water as primary factors [97]. In a study of *Campylobacter* contamination of thigh/breast of poultry, and ovine, bovine and porcine liver, in an area surrounding a reference laboratory in the UK, the *C. coli* strain characteristics of isolates from porcine liver were not comparable to the human isolates, leading the authors to postulate that pork is a relatively minor contributor to human campylobacteriosis [106]. While the most predominant serotype identified among

human isolates was found in all food sources, the authors noted that this serotype was the least frequently isolated from pig liver as compared to the other types of meats surveyed. Although the Nielson *et al.* suggests that cattle are a major source of human infections due to serotype overlap, other epidemiological studies do not identify beef consumption as a major risk factor for campylobacteriosis in humans [107,108,109].

Munroe *et al.* [110] isolated *C. jejuni* and *C. coli* from feces of chicken, cattle and swine. Whereas 96% of the chicken isolates were identified as *C. jejuni* serotypes frequently isolated from human cases of enteritis, the *Campylobacter* isolated from swine feces were 97% *C. coli* and did not belong to serotypes common to human isolates [111]. Similarly, Nielson *et al.* [112] observed that 94% of human *Campylobacter* diarrhea isolates were *C. jejuni,* and *C. coli* was isolated from patients at only a 6% rate. The investigators concluded that swine were not an important source of human *Campylobacter* infections because of the differences in serotypes between human disease and swine fecal isolates [112]. The authors reported overlap of serotypes of isolates from humans and those from live poultry and from live cattle among Danish isolates. The authors suggested that poultry and cattle are sources of campylobacter in humans, noting the relatively high prevalence of *Campylobacter* at retail, and suggesting that other foods should be considered. The authors reported overlap of serotypes of isolates from humans and those from live poultry and from live cattle.

Based on these epidemiological studies and reviews, the exposure of humans to *Campylobacter* via beef and pork is relatively low.

2.3. Exposure assessment for cattle

2.3.1. Qualitative ranking of human consumption of beef in the US

In Guidance #152 [1], beef consumption in the United States is qualitatively ranked as "High" (64.5 lb/capita/year), based on the most recent data available from the USDA Economic Research Service (ERS) [113].

2.3.2. Qualitative ranking of Campylobacter contamination of beef

Guidance #152 [1] lists qualitative rankings for beef contamination by *Campylobacter,* based on the USDA Food Safety Inspection Service (FSIS) Nationwide Beef Microbiological Baseline Data collection program [114,115,116]. These rankings are "Low" for ground beef (0% contamination [114]), cows/bulls (1% contamination [115]) and steers/heifer (4% contamination [116]) samples. These contamination results are consistent with recent data generated by the FDA/CVM in the new NARMS sentinel site studies of retail meat [7]. Among the 642 and 809 ground beef samples tested for *Campylobacter* for years 2002 (6 sentinel sites) and 2003 (8 sentinel sites), only 1% of the samples from retail ground beef each year yielded positive results for *Campylobacter.* In Guidance #152, Table 5 provides one means to qualitatively rank human exposure to *Campylobacter,* based on these national data.

2.3.3. Overall Beef Exposure Assessment Conclusion: "Medium"

The application of the USDA ERS beef consumption data (ranked "High" per capital consumption), the beef contamination rate data (ranked "Low"), based on FSIS data [114,115,116], and the NARMS Sentinel Site Retail Meat contamination rate data [7] to this ranking process yields an overall exposure assessment of "Medium" for human exposure to *Campylobacter* through consumption of beef, based on the process described in Table 5 of Guidance #152 (Figure 1).

2.4. Exposure assessment for swine

2.4.1. Qualitative ranking of human consumption of pork in the US

In Guidance #152 [1], pork consumption in the United States is qualitatively ranked as "High" (48.2 lb/capita/year), based on the most recent data available from the ERS [113].

2.4.2. Qualitative ranking of Campylobacter contamination of pork

2.4.2.1. Default contamination values based on pork carcass data

Guidance #152, qualitatively ranks the contamination rate of pork by *Campylobacter* as "High", based on the USDA FSIS Baseline Data Collection Program for Market Hogs in 1995-6 [1,43]. The FSIS sampling in this baseline study included only carcasses sampled after 12 hours chilling, and before completion of the pork processing steps for retail distribution. These processing are very detrimental to the survival of *Campylobacter* as detailed below (Section 2.4.3.3, 2.4.3.4, 2.4.3.5). Thus the baseline study used in Guidance #152 to estimate pork contamination rates by *Campylobacter* substantially overestimates the exposure of humans to *Campylobacter* by pork at retail. This is corroborated by retail meat studies as discussed below, as well as the epidemiological studies cited in Sections 2.2 and 2.4.3.5.

2.4.3. Review of Campylobacter contamination in carcass and pork meat

2.4.3.1. Campylobacter in swine

C. coli, but not *C. jejuni,* is frequently found in the gastrointestinal tracts of swine. Surveys show 60-100% detection of *Campylobacter* species in feces of swine herds in various parts of the world [102,117,118]. In a national swine study coordinated by the United States NAHMS program, the predominant *Campylobacter* species isolated from 1057 fecal samples was *C. coli,* detected in 69% of the fecal samples (n=1057) while *C. jejuni* was detected in only 0.3% of samples [102, 119]. Burch estimated from a review various surveys of swine, mainly in Europe, that 96% of *Campylobacter* isolated from pig feces was *C. coli,* and only 4% were *C. jejuni* [92]. Nielson *et al.* observed that among 316 fecal samples collected from 19 swine slaughterhouses in Denmark, 46% were positive for *Campylobacter,* 95% of which were speciated as *C. coli,* and only 4% as *C. jejuni* [112].

2.4.3.2. Pork carcass contamination

Given the relatively high prevalence rates of *C. coli* observed in studies of swine feces, it is not surprising that carcass data would also suggest a high prevalence of *Campylobacter.* In a

study conducted in Iowa in1999, the average carcass contamination level was 9% (Table 10 of Reference [102]). In the 1995-1996 National Pork Microbiological Baseline Data Collection Program for Market Hogs, *Campylobacter* was detected in 32% of 2112 samples of carcasses [120], with detection levels of $\leq$ 0.03 cells/cm^2 of surface area sampled (1 cm thick samples) (Table 6).

Table 6. *Campylobacter* distribution among raw pork carcass surface samples tested positive for *Campylobacter* in the US Nationwide Pork Microbiological Baseline Data Collection Program.

Quantitative cell count range* (MPN/cm^2)*	Projected MPN/200 cm^2	No Samples* testing positive at this level of pathogen burden*
$\leq$0.03	$\leq$6	508
0.03 - 0.30	6-60	113
0.31-3.0	60-600	13
3.1-30.0	600-6000	4
>30.0§	>6000	1
Total positive samples		639
Total negative samples		1473
Total samples tested		2112

The National Research Council and Institute of Medicine reported that 10% of carcasses were scored positive for *Campylobacter* (Table 5-3 of Reference [121]). In a Norwegian study, 36% of swine carcasses were positive in 2002 (Table 5 of Reference [117]). The Norwegian isolates were predominantly *C. coli;* and *C. jejuni* was not isolated from swine carcass (Table 4 of Reference [117]). In a Canadian study of swine at slaughter, *C. coli*, *C. jejuni* and *C. laridis* isolated from swine feces accounted for 97%, 2% and 1% of the isolates, respectively [122], and the isolation rates of the same three *Campylobacter* species were lower from diaphragm specimens of the carcasses before cold storage, 20%, 2%, and 1%, respectively. These studies all corroborate the relatively high pork carcass contamination rates, but the detection limits for studies will vary, and in the case of the US survey, the limit of detection was very low.

2.4.3.3. Campylobacter *in carcasses does not represent exposure to consumers*

The carcass contamination rates recorded in the National Pork Microbiological Baseline Data Collection Program in market hogs [120] represent a high estimate of actual consumer exposure rates to *Campylobacter.* The carcasses in this study were sampled after 12 h cooler chilling, which marked a natural break in a pork processing operation, and provided a window of time for convenient sampling by the USDA FSIS. Typical hog slaughter plants use a scalding procedure to remove all hair from the carcass, leaving the skin intact on the carcass during this stage of the chilling process. The skin still remains on the carcass during this sampling time. *Campylobacter* species were recovered from 32% of the 2112 carcasses

sampled using a selective enrichment procedure from a 60 cm^2 composite sample of the carcass surface (1 cm deep cuts for sampling) from samples obtained from the jowl, belly and ham, followed by a selective, bacterial enrichment procedure for *Campylobacter*. If a sample tested positive it was then reanalyzed using a standard mean probable number bacteriological enumeration procedure to determine the number of organisms per cm^2 of carcass surface area (Table 6). These samples were taken before removal of skin. Typically after chilling (the sampling time for the *Campylobacter* survey), the carcass is broken into primal cuts such as the belly, shoulder, ham and loin. The primal cuts are further processed by removal of the skin and excess fat. The skin and fat do not accompany either the primal or commercial fresh pork products into the market place. Fresh pork skin is typically not consumed as an edible tissue. Most skin and associated subcutaneous fat trimmed from the carcass are rendered at high temperature to produce lard and other cooked products. During and after final processing of pork, primal cuts are exposed to cold and aerobic environments that are hostile to the survival of *Campylobacter* [98,102,123]. Exposure to air and removal of the skin should greatly decrease exposure to *Campylobacter* for reasons described above. This is supported by both the low prevalence of Campylobacter on beef carcass (hide removed before sampling, Section 2.3.1) and low prevalence of *Campylobacter* contamination (<5%) of fresh pork products sampled at retail (See Section 2.4.3.5).

2.4.3.4. Low survival rates of Campylobacter *survival from carcass to retail*

Survival rates of *Campylobacter* are adversely impacted by exposure to air, low temperatures (freezing) and desiccation. This may explain the low contamination rates in retail pork (Section 2.4.3.5). Several review articles describe the inability of these organisms to survive the environmental conditions encountered on swine carcasses [98,102,124], demonstrating the sensitivity of *Campylobacter* to oxygen and pH extremes, their sensitivity to drying by forced air chilling and freezing. These conclusions are supported by field observations [117,125,126]. Therefore, because of *Campylobacter* environmental sensitivity, it is expected that observed carcass contamination rates in the sampling scheme used by the USDA Food Safety Inspection Service would be lower than the contamination rates observed at retail.

These conclusions are supported by field observations. In one study, the contamination rates of *Campylobacter* on carcasses dropped from 9% after slaughter, to 0% after chilling [125]. In another study [117], 56.7% of carcasses were positive for *C. coli*, but after blast-freezing only 1.7% of the same carcasses were positive. While *Campylobacter* was detected in 100% and 80% of rectal and colonic samples, respectively [126] in a recent study by the USDA of 30 swine carcasses (sub sample of 360) through processing, only 33% of the carcasses were positive for *Campylobacter* immediately after exsanguination, 0% after polishing, 7% before chilling and 0% after overnight chilling [126]. *C. jejuni* represented only 1% of the *Campylobacter* isolates.

In a study where *C. coli* was inoculated onto pork meat at 3000 CFU/cm^2 and subjected to blast chilling, counts were reduced to undetectable levels even when bacterial growth enrichment methods were used in attempts to recover *Campylobacter* [126].

Thus, the likelihood of *Campylobacter* survival post carcass sampling is low, and would help explain why retail meat sampling indicate low prevalence rates for *Campylobacter*.

2.4.3.5. Retail pork meat contamination

The carcass contamination levels summarized above for *Campylobacter* are much higher than what is observed in raw pork products at retail. In a survey of 59 retail meat stores in the Washington DC area in 2001, pork products had a *Campylobacter* prevalence rate of 1.7% [77] among 181 samples surveyed. In a Netherlands survey in 2001, 0% of 524 pork samples and 0.4% of 255-minced pork/beef samples tested positive [127]. In a study conducted at Colorado State University wherein meat samples were collected from 24 retail stores across the United States, 1.3% of 384 samples were positive [128,129]. The highest contamination rates occurred in ground pork products at 3.1% [128,129]. The authors of the latter study concluded that *Campylobacter* was the least frequent pathogen found on retail pork samples [129]. Alketruse *et al*. [96] noted lowest contamination rates in pork samples compared to beef and poultry. In the most recent preliminary data reported for the NARMS Sentinel Site Retail Meat surveys [7], the prevalence of *Campylobacter* isolated from pork chops was 1% in survey years 2002 (n=613 pork chops sampled, 6 sentinel sites) and 2003 (n=829 pork chops sampled, 8 sentinel sites).

Collectively, these studies repeatedly demonstrate that although there can be high prevalence rates of *Campylobacter* in swine feces and soon after slaughter on carcass with skin on, the organism does not persist through the pork meat processing and survive to retail.

2.4.3.6. Qualitative ranking of Campylobacter contamination of pork as "Low"

Guidance #152 [1] lists a *Campylobacter* contamination rate of 32% in market hogs (Table B3 of Appendix B of Reference [1]). While these numbers might be expected, given the high prevalence of *C. coli* in live swine, the 32% value is a very high overestimate of the contamination rates in raw pork meat at retail, based on the data above. As summarized in Section 2.4.3.2, the actual abundance of *Campylobacter* cells in these positive samples was very low. Most of these positive samples had quantitative cell levels $\leq$ 0.03 *Campylobacter* cells per cm^2 as estimated by the most probable number method (Table 6). Furthermore, data summarized in Sections 2.4.3.3, 2.4.3.4, and 2.4.3.5 support the low contamination rates at retail.

Qualitatively, the risk of ingestion of *Campylobacter* is “Low”, without further processing or cooking. The survival characteristics of *Campylobacter* in pasteurized milk, and cooked meat suggest that *Campylobacter* is rapidly inactivated by minimal pasteurization [130] (60°C for 80 sec) or cooking meat to an internal temperature of 70°C [131]. In addition, curing and cooking of processed pork products will also help eliminate contamination by most organisms including *Campylobacter*.

This qualitative ranking of “Low” exposure is further corroborated by the fact that *Campylobacter* isolated from humans is generally *C. jejuni*, and not the predominant *C. coli* organisms typically isolated from swine, and that epidemiologically, pork meat is not listed as a major risk factor for *Campylobacter*.

2.4.3.7. Qualitative ranking of Campylobacter contamination of pork is "Low"

Guidance #152 [1] contains non-binding recommendations regarding risk assessment. According to Guidance #152, the qualitative determination that *Campylobacter* of pork is "High". The Sponsor proposes that this default contamination rate is an overestimate of the true overall exposure assessment to the consumer, based on the following application of the data summarized in Exposure Assessment Section 2.4.2). Rather, the qualitative prevalence ranking of *Campylobacter* in pork derived food commodities is "Low" because studies measure contamination rates much less than 5% in pork retail meats [7,127,128,129] as discussed above.

2.4.4. Overall Pork Exposure Assessment Conclusion: "Medium"

The application of the ERS pork consumption data (ranked "High" per capita consumption) and the retail pork contamination rate (based on the literature summarized in Section 2.4.3, including the NARMS Sentinel Site Retail Meat contamination rate data for pork [7]) to this ranking process yields an overall exposure assessment of "Medium" for human exposure to *Campylobacter* through consumption of pork, based on the process described in Table 5 of Guidance #152 (Figure 1).

3. CONSEQUENCE ASSESSMENT

The Consequence Assessment of macrolides in human medicine is "Critically Important " because macrolides are used for treatment of the foodborne pathogen, *Campylobacter,* associated with food-producing animals and because they are important for use in treatment of Legionnaire's disease, and prophylaxis and therapy for serious disease due to *Mycobacterium avium* Complex (MAC) and *Mycobacterium avium-intracellulare* (MAI), as presented in Appendix A of Guidance #152 [1].

4. OVERALL QUALITATIVE RISK ESTIMATION

4.1. Release Assessment Summary

The Release Assessment is ranked as "Low" probability that macrolide-resistant *Campylobacter* will emerge or be selected as consequence of the proposed use of tulathromycin, as outlined in Section 1.

4.2. Exposure assessment summary

For cattle and for swine, the Exposure Assessment is a "Medium" probability that humans will be exposed to *Campylobacter* as a result of exposure to food products derived from cattle or swine, as outlined in Section 2.

4.3. Consequence assessment summary

The Consequence Assessment of macrolides in human medicine is "Critically Important " because macrolides are used for treatment of the foodborne pathogen, *Campylobacter,* associated with food-producing animals and because they are important for use in treatment of Legionnaire's disease, and prophylaxis and therapy for serious disease due to

Mycobacterium avium Complex (MAC) and *Mycobacterium avium-intracellulare* (MAI), as presented in Appendix A of Guidance #152 [1].

4.4. Overall qualitative risk estimation is “High” for a “Critically Important” drug

The three qualitative assessments above ("Low" Release Assessment; "Medium" Exposure Assessment, "Critically Important" Consequence Assessment) can be integrated consistent with Table 6 of Guidance #152 [1].

All "Critically Important Drugs" result is an Overall Risk Estimation of "High" regardless of what the ranking result is for the Release and Exposure Assessments.

5. RISK MANAGEMENT CONSIDERATIONS

5.1. Inherent properties of tulathromycin

There are inherent characteristics of tulathromycin and its proposed use that lower the potential for concern for selection of macrolide-resistant *Campylobacter*. First, tulathromycin antibacterial activity in the colonic content and feces is limited due to the pH in these microbial environments, lessening selective pressure during the transient period in which it occurs in the gut post dose. Second, macrolide resistance occurs in *Campylobacter* by chromosomal mutation and not by gene-acquisition. Third, macrolide resistance in *Campylobacter* isolates from humans has remained $\leq$ 3% with no obvious trends over time in the-NARMS surveys despite widespread macrolide use in humans, companion animals and food animals. Fourth, the prevalence of any *Campylobacter* in United States pork and beef at retail is low (0-5%) and the microbiological, molecular, epidemiological, and historical data base supports that pork and beef is not a major risk factor for *Campylobacter* causing disease in humans.

5.2. Extent of use

The extent of use limitations listed in Table 7 of Guidance #152 (Figure 2) suggest that the extent of use is considered "Low" if the drug is intended for administered to individuals, and the duration of effective dose is <21 days. Therefore, tulathromycin (a branded proprietary product) use in the treatment of BRD and SRD by individual animal injection is consistent with a "Low" extent of use. Extralabel use may be considered under the guidance of a veterinarian, within the context of a valid veterinarian/client/patient relationship. Alternative drug therapies (tilmicosin, ceftiofur, florfenicol) are available for comparable indications.

5.3. Approval and Risk Management Steps

Examples of potential risk management steps for "Category I" drugs having an overall "High" Risk Estimation are listed in Table 8 of FDA Guidance #152 [1], copied below.

Figure 3 Table 8 of Guidance #152 [1]Examples of potential risk management steps associated with the approval of antimicrobial new animal drugs in food-producing animals based on the level of risk (high, medium, or low).

Approval conditions	Category 1 (High)	Category 2 (Medium)	Category 3 (Low)
Marketing Status[1]	Rx	Rx/VFD	Rx/VFD/OTC
Extra-label use (ELU)	ELU Restrictions	Restricted in some cases[3]	ELU permitted
Extent of use[2]	Low	Low, medium	Low, medium, high
Post-approval monitoring (e.g., NARMS)	Yes	Yes	In certain cases
Advisory committee review considered	Yes	In certain cases[3]	No

[1]Prescription (Rx), Veterinary Feed Directive (VFD), Over-The-Counter (OTC)
[2]See Table 7 for characterization of extent of use
[3]These risk management steps may be appropriate for certain Category 2 drugs that were ranked critically important for consequence assessment **and** ranked "high" for release **or** exposure assessment

The Sponsor proposes that tulathromycin should be approved as a veterinary prescription product. The extent of use will be inherently low, based on the proposed use and parenteral single dosage. This document is submitted as a component of the Advisory Committee review. Extralabel use restrictions are not required for this approval, because macrolides have been approved and used extensively for a variety of indications in poultry, swine, cattle and other animal species for decades. The proposed uses of tulathromycin are consistent with judicious use principles outlined by the FDA/CVM in collaboration with the American Veterinary Medical Association [59,60]. Furthermore, the database reviewed and accepted by the FDA/CVM supports that tulathromycin has a similar mechanism of action as conventional macrolides approved for animal use, and has resistance and cross-resistance profiles common to approved macrolides. Tulathromycin has no more potential to select for unique resistance mechanisms that would compromise macrolide use in humans than any of the macrolides already used in animal medicine and that the selection pressure of existing macrolides use in animals is substantially more than any incremental pressure that use of this product might provide.

6. CONCLUSION

With respect to microbial safety considerations, the proposed label uses of tulathromycin include management considerations of prescription status, inherent low extent of use due to parenteral single dose administration, and Advisory Committee review. Macrolide resistance in *Campylobacter* is currently monitored in the NARMS program. Therefore, with these management considerations, approval of the proposed indications for injectable tulathromycin in cattle and swine poses no appreciable risk to public health with respect to microbial food safety.

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